# COSEWIC Assessment and Status Report

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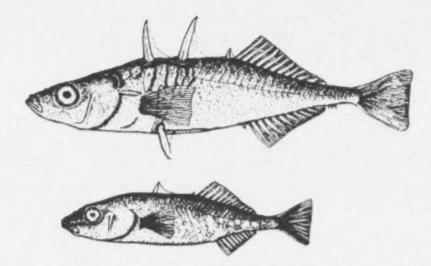
# **Giant Threespine Stickleback**

Gasterosteus aculeatus

# **Unarmoured Threespine Stickleback**

Gasterosteus aculeatus

in Canada



SPECIAL CONCERN 2013

COSEWIC

Committee on the Status of Endangered Wildlife in Canada



COSEPAC

Comité sur la situation des espèces en péril au Canada

COSEWIC status reports are working documents used in assigning the status of wildlife species suspected of being at risk. This report may be cited as follows:

COSEWIC. 2013. COSEWIC assessment and status report on the Giant Threespine Stickleback Gasterosteus aculeatus and the Unarmoured Threespine Stickleback Gasterosteus aculeatus in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. xiv + 62 pp. (www.registrelep-sararegistry.gc.ca/default\_e.cfm).

Previous report(s):

Reimchen, T.E. 1984. COSEWIC status report on the Charlotte Unarmoured Stickleback *Gasterosteus* sp. in Canada Committee on the Status of Endangered Wildlife in Canada. Ottawa. 13 pp.

Moodie, G.E.E. 1980. COSEWIC status report on the Giant Sticklebank *Gasterosteus sp.* in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. 6 pp.

#### Production note:

COSEWIC would like to acknowledge Dr. Jennifer Gow for writing the status report on the Giant Threespine Stickleback and Unarmoured Threespine Stickleback, *Gasterosteus aculeatus*, in Canada, prepared under contract with Environment Canada. This report was overseen and edited by Dr. Eric Taylor, Co-chair of the COSEWIC Freshwater Fishes Specialist Subcommittee.

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Également disponible en français sous le titre Évaluation et Rapport de situation du COSEPAC sur L'épinoche à trois épines géante (Gasterosteus aculeatus) et L'épinoche à trois épines lisse (Gasterosteus aculeatus) au Canada.

Cover illustration/photo

Giant Threespine Stickleback and the Unarmoured Threespine Stickleback — Giant Threespine Stickleback (upper) and Unarmoured Threespine Stickleback (lower) Gasterosteus aculeatus. From Moodie and Reimchen (1976) by permission.

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#### Assessment Summary - November 2013

#### Common name

Giant Threespine Stickleback

#### Scientific name

Gasterosteus aculeatus

#### Status

Special Concern

#### Reason for designation

This freshwater stickleback is of unusually large size and is currently known to exist in two small lakes that are in relatively remote areas. The populations could, however, quickly become Endangered if invasive species were to be introduced as has been observed in other stickleback populations.

### Occurrence

British Columbia

#### Status history

Designated Special Concern in April 1980. Status re-examined and confirmed in November 2013.

#### Assessment Summary - November 2013

#### Common name

Unarmoured Threespine Stickleback

#### Scientific name

Gasterosteus aculeatus

#### Status

Special Concern

#### Reason for designation

This morphologically distinctive small-bodied freshwater fish is currently known to exist in only three very small lakes that are in a relatively remote area. The populations could, however, quickly become Endangered if invasive species were to be introduced as has been observed in other stickleback populations.

# Occurrence

British Columbia

#### Status history

Designated Special Concern in April 1983. Status re-examined and confirmed in November 2013.



# Giant Threespine Stickleback Gasterosteus aculeatus

# Unarmoured Threespine Stickleback Gasterosteus aculeatus

# Wildlife Species Description and Significance

The Giant Threespine Stickleback has a mean adult standard length (SL) exceeding 75 mm, being almost twice the length of most other freshwater Threespine Stickleback. Although not unique, several other morphological features also set it apart from the "typical" freshwater form; it has a more streamlined shape; more gill rakers and robust body armour; and has an unusual colouration. The two confirmed populations of the Giant Threespine Stickleback appear to have evolved independently from one another, and each one appears to be at least partially reproductively isolated from the Threespine Stickleback that inhabits the streams connected to its lakes. The Unarmoured Threespine Stickleback is one of a few populations across the global range of Threespine Stickleback that exhibit extensive loss of defensive spines. Its three confirmed occurrences are characterized by the loss of one or more spines in the majority of fish. They appear to have evolved independently from one another. Both species contribute to the extensive morphological variation displayed by Threespine Stickleback from Haida Gwaii, and have intrinsic value as significant prey items in their ecosystems. They continue to provide significant insights into the processes involved in evolutionary change.

#### Distribution

The known global range of the Giant Threespine Stickleback is restricted to just two lakes (Drizzle and Mayer). The three lakes (Boulton, Rouge and Serendipity) on Haida Gwaii (formerly Queen Islands) harbouring the Unarmoured Threespine Stickleback represent a significant proportion of the Canadian and global range of Unarmoured Threespine Stickleback. Future analysis of other purported instances may confirm more occurrences both of Giant and Unarmoured Threespine Stickleback.

#### Habitat

The Giant Threespine Stickleback is known to occur in two lakes that range in size from about 100 to 600 ha. Both lakes are "tea-stained" in colour and acidic, typically containing fish-eating fishes and birds. It is thought to have evolved its distinct morphology at least in part as a result of adaptation to these predators. In contrast, the Unarmoured Threespine Stickleback is restricted to small (< 20 ha), shallow bog ponds which have no fish-eating fish and few predatory birds. It is thought to have evolved its distinguishing loss of spines at least in part as a result of adaptation to a relative lack of predation by vertebrates, and predation by large invertebrates that grapple onto spines of stickleback. Both species most likely share other habitat requirements in common with other lake-dwelling Threespine Stickleback, such as sustained lake productivity, absence of invasive species, and maintenance of natural aquatic plants for nesting and juvenile rearing.

# Biology

The reproductive biology of the Giant Threespine Stickleback is similar to other freshwater Threespine Stickleback, but it exhibits several striking deviations: its loss of male red breeding colouration; its production of a greater number of eggs than is usual; and its extended life span. It is tolerant of low calcium levels and pH, and heavily tanninstained waters. It is largely confined to its two separate lakes, with very little migration between, or interbreeding with, the Threespine Stickleback that inhabits the streams connected to its lakes. The reproductive biology of the Unarmoured Threespine Sticklebacks is likely similar to other freshwater Threespine Stickleback. It is exceptionally tolerant of acidic waters. Each population is geographically isolated from the other and from other Threespine Stickleback.

# **Population Sizes and Trends**

A population size of 75,000 adults was estimated for the Giant Threespine Stickleback in Drizzle Lake using mark-recapture methods. Expert opinion estimates the number of adults in Mayer Lake to exceed 100,000. Total subpopulation sizes for Unarmoured Threespine Stickleback have been crudely estimated to be 350,000 for Boulton Lake, 17,500 for Rouge Lake, and 22,000 for Serendipity Lake. There has been no systematic monitoring of abundance of the Giant or Unarmoured Threespine Stickleback so population trends are unknown. Based on general observations, population sizes are assumed to be stable.

# **Threats and Limiting Factors**

Invasive species are the greatest potential threat to both the Giant and Unarmoured Threespine Stickleback. Other anthropogenic disturbances in habitats required by these two species most likely present another significant threat. Specific potential threats to the Giant Threespine Stickleback come from a decline in predation pressure from Coastal Cutthroat Trout (e.g., from overfishing) and/or Common Loon (e.g., from recreational disturbance). Specific potential threats to the Unarmoured Threespine Stickleback include predation from gape-limited predators resulting from the introduction of predatory fish such as Coastal Cutthroat Trout, the impact of rural and industrial activities around Boulton Lake, and drainage of Serendipity Lake caused by coastal erosion.

# Protection, Status, and Ranks

Currently, both the Giant and Unarmoured Threespine Stickleback are designated as Special Concern by COSEWIC. The Giant Threespine Stickleback is listed as Critically Imperilled globally, nationally and provincially by NatureServe, and its General Status at the Canada and provincial levels was ranked as Sensitive in 2000. It is "red-listed" by the Conservation Data Centre and BC Ministry of Environment, and is ranked 1 under Goal 1 and Goal 3 of the BC Conservation Framework. The Unarmoured Threespine Stickleback is listed as Imperilled both nationally and provincially by NatureServe. It is "red-listed" by the Conservation Data Centre and BC Ministry of Environment, and is ranked 1 under Goal 1 of the BC Conservation Framework.

# **TECHNICAL SUMMARY 1**

Gasterosteus aculeatus
Giant Threespine Stickleback
Epinoche à trois épines géante
Range of occurrence in Canada (province/territory/ocean): British Columbia

# **Demographic Information**

Generation time (usually average age of parents in the population; indicate if another method of estimating generation time indicated in the IUCN guidelines (2008) is being used)	2-3 yrs
Is there an observed, inferred, or projected continuing decline in number of mature individuals?  Population size estimates have been made only once several decades ago, but there is no reason to suspect significant declines in abundance	Unknown, but probably not
Estimated percent of continuing decline in total number of mature individuals within 5 years.  Probably stable	Unknown
Suspected percent reduction or increase in total number of mature individuals over the last 10 years.  Probably stable	Unknown
Suspected percent reduction or increase in total number of mature individuals over the next 10 years.  Probably stable	Unknown
Suspected percent reduction or increase in total number of mature individuals over any 10 year period, over a time period including both the past and the future.  Probably stable	Unknown
Are the causes of the decline clearly reversible and understood and ceased?	Not applicable, no suspected decline
Are there extreme fluctuations in number of mature individuals?	Unknown, but probably not

# **Extent and Occupancy Information**

Estimated extent of occurrence	63 km²
Index of area of occupancy (IAO) (Always report 2x2 grid value).	52 km²
Is the population severely fragmented?	No
Number of locations*	Two
Is there an observed, inferred, or projected continuing decline in extent of occurrence?	No
Is there an observed, inferred, or projected continuing decline in index of area of occupancy?	No
Is there an observed, inferred, or projected continuing decline in number of populations?	No
Is there an observed, inferred, or projected continuing decline in number of locations*?	No

Is there an observed, inferred, or projected continuing decline in area, extent and/or quality of habitat?	No
Are there extreme fluctuations in number of populations?	No
Are there extreme fluctuations in number of locations*?	No
Are there extreme fluctuations in extent of occurrence?	No
Are there extreme fluctuations in index of area of occupancy?	No

## Number of Mature Individuals (in each population)

Population Estimates are based on various techniques (mark-recapture, nest densities) and are considered approximate only. Notwithstanding these caveats, total adult population sizes are likely in excess of several tens of thousands per lake.	N Mature Individuals
Mayer Lake	~75,000
Drizzle Lake	> 100,000
Total	> 175,000

# **Quantitative Analysis**

Probability of extinction in the wild is at least [20% within 20 years or 5	Not available	
generations, or 10% within 100 years].		

# Threats (actual or imminent, to populations or habitats)

Introduction of invasive species; decline in predation pressure from Coastal Cutthroat Trout and/or Common Loon populations

# Rescue Effect (immigration from outside Canada)

Status of outside population(s)?	None exist
Is immigration known or possible?	NA
Would immigrants be adapted to survive in Canada?	NA
Is there sufficient habitat for immigrants in Canada?	NA
Is rescue from outside populations likely?	No

# **Data-Sensitive Species**

Is this a data-sensitive species?	No

# **Status History:**

COSEWIC: Designated Special Concern in April 1980. Status re-examined and confirmed in November 2013.

#### Status and Reasons for Designation:

Status:	Alpha-numeric code:
Special Concern	Not applicable

## Reason for Designation:

This freshwater stickleback is of unusually large size and is currently known to exist in two small lakes that are in relatively remote areas. The populations could, however, quickly become Endangered if invasive species were to be introduced as has been observed in other stickleback populations.

#### Criterion A

Not applicable. No evidence of decline.

#### Criterion B

Not applicable. Nearly meets Endangered for B1 (EO = 63 km²), B2 (IAO = 52km²) and sub-criterion a (known locations = 2), but none of the other sub-criteria.

#### Criterion C:

Not applicable. Exceeds thresholds and no evidence of declines.

# Criterion D:

Not applicable. Exceeds thresholds.

#### Criterion E:

Not applicable. Data necessary for evaluation not available.

# **TECHNICAL SUMMARY 2**

Gasterosteus aculeatus

Unarmoured Threespine Stickleback

Épinoche à trois épines lisse

Range of occurrence in Canada (province/territory/ocean): British Columbia

# **Demographic Information**

Generation time (usually average age of parents in the population; indicate if another method of estimating generation time indicated in the IUCN guidelines (2008) is being used)	1-2 yrs
Is there an observed, inferred, or projected continuing decline in number of mature individuals?  Population size estimates have been made only once several decades ago, but there is no reason to suspect significant declines in abundance	Unknown, but probably not
Estimated percent of continuing decline in total number of mature individuals within 5 years. Probably stable	Unknown
Suspected percent reduction or increase in total number of mature individuals over the last 10 years.  Probably stable	Unknown
Suspected percent reduction or increase in total number of mature individuals over the next 10 years.  Probably stable	Unknown
Suspected percent reduction or increase in total number of mature individuals over any 10 year period, over a time period including both the past and the future.  Probably stable	Unknown
Are the causes of the decline clearly reversible and understood and ceased?	Not applicable, no suspected decline
Is there an observed, inferred, or projected continuing decline in number of mature individuals?  Population size estimates have been made only once several decades ago, but there is no reason to suspect significant declines in abundance	Unknown, but probably not

# **Extent and Occupancy Information**

Estimated extent of occurrence	124 km²
Index of area of occupancy (IAO) (Always report 2x2 grid value).	20 km²
Is the population severely fragmented?	No
Number of locations*	Three
Is there an observed, inferred, or projected continuing decline in extent of occurrence?	No
Is there an observed, inferred, or projected continuing decline in index of area of occupancy?	No
Is there an observed, inferred, or projected continuing decline in number of populations?	No
Is there an observed, inferred, or projected continuing decline in number of locations*?	No

Is there an inferred or projected continuing decline in extent and/or quality of habitat?	Probably not
Are there extreme fluctuations in number of populations?	No
Are there extreme fluctuations in number of locations*?	No
Are there extreme fluctuations in extent of occurrence?	No
Are there extreme fluctuations in index of area of occupancy?	No

### Number of Mature Individuals (in each population)

Population Estimates are based on various techniques (mark-recapture, nest densities) and are considered approximate only. Notwithstanding these caveats, total adult population sizes are likely in excess of 10,000 per lake.	N Mature Individuals
Boulton Lake	~350,000
Rouge Lake	~17,500
Serendipity Lake	~22,000
Total	389,500

# **Quantitative Analysis**

Probability of extinction in the wild is at least [20% within 20 years or 5	Not available	
generations, or 10% within 100 years].		

# Threats (actual or imminent, to populations or habitats)

Introduction of invasive species, especially predatory fish such as Coastal Cutthroat Trout; rural and industrial activities; drainage caused by coastal erosion

#### Rescue Effect (immigration from outside Canada)

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Status of outside population(s)?	None exist
Is immigration known or possible?	NA
Would immigrants be adapted to survive in Canada?	NA
Is there sufficient habitat for immigrants in Canada?	NA
Is rescue from outside populations likely?	No

#### **Data-Sensitive Species**

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Is this a data-sensitive species?	No

# **Status History:**

COSEWIC: Designated Special Concern in April 1980. Status re-examined and confirmed in November 2013.

# Status and Reasons for Designation

Status:	Alpha-numeric code:
Special Concern	Not applicable

### Reason for Designation:

This morphologically distinctive small-bodied freshwater fish is currently known to exist in only three very small lakes that are in a relatively remote area. The populations could, however, quickly become Endangered if invasive species were to be introduced as has been observed in other stickleback populations.

#### Criterion A:

Not applicable. No evidence of decline.

#### Criterion B:

Not applicable. Nearly meets Endangered for B1 (EO = 124 km²), B2 (IAO = 20km²) and sub-criterion a (known locations = 2), but none of the other sub-criteria.

#### Criterion C:

Not applicable. Exceeds thresholds and no evidence of declines.

#### Criterion D:

Not applicable. Exceeds thresholds.

#### Criterion E:

Not applicable. Data necessary for evaluation not available.

## **PREFACE**

The Giant and Unarmoured Threespine Sticklebacks were last assessed by COSEWIC as Special Concern in 1980 and 1983, respectively, and both are listed on Schedule 3 of the Species at Risk Act as such. Several attempts to update status reports for these wildlife species were forestalled by efforts to gain a better understanding of the tremendous diversity with the Gasterosteus aculeatus species complex particularly as it pertains to identifying priorities for conservation. Since the late 1990s there has been a tremendous surge in the use of Threespine Stickleback, in all its forms, as a model species in studies of evolutionary process. This has resulted in a much greater understanding of the geographic and ecological factors responsible for the evolution of diversity in this complex and, most recently, a better understanding of the evolution of the Giant and Unarmoured Threespine Sticklebacks. Although the Giant and Unarmoured Threespine Sticklebacks are distinct designatable units within the G. aculeatus species complex, they are assessed together given their common occurrence on Haida Gwaii and because they share some threats. In addition, the major threat to both sticklebacks is the potential for invasive, predatory fishes to eliminate populations which has been recorded in other sticklebacks. There has been significant new knowledge acquired since the last status report on the occurrence and relative probabilities of establishment of invasive species in the range of both sticklebacks.



#### **COSEWIC HISTORY**

The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) was created in 1977 as a result of a recommendation at the Federal-Provincial Wildlife Conference held in 1976. It arose from the need for a single, official, scientifically sound, national listing of wildlife species at risk. In 1978, COSEWIC designated its first species and produced its first list of Canadian species at risk. Species designated at meetings of the full committee are added to the list. On June 5, 2003, the *Species at Risk Act* (SARA) was proclaimed. SARA establishes COSEWIC as an advisory body ensuring that species will continue to be assessed under a rigorous and independent scientific process.

#### COSEWIC MANDATE

The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) assesses the national status of wild species, subspecies, varieties, or other designatable units that are considered to be at risk in Canada. Designations are made on native species for the following taxonomic groups: mammals, birds, reptiles, amphibians, fishes, arthropods, molluscs, vascular plants, mosses, and lichens.

#### COSEWIC MEMBERSHIP

COSEWIC comprises members from each provincial and territorial government wildlife agency, four federal entities (Canadian Wildlife Service, Parks Canada Agency, Department of Fisheries and Oceans, and the Federal Biodiversity Information Partnership, chaired by the Canadian Museum of Nature), three non-government science members and the co-chairs of the species specialist subcommittees and the Aboriginal Traditional Knowledge subcommittee. The Committee meets to consider status reports on candidate species.

#### DEFINITIONS (2013)

	(2010)	
Wildlife Species	A species, subspecies, variety, or geographically or genetically distinct population of animal plant or other organism, other than a bacterium or virus, that is wild by nature and is either native to Canada or has extended its range into Canada without human intervention and has been present in Canada for at least 50 years.	,
Extinct (X)	A wildlife species that no longer exists.	
Extirpated (XT)	A wildlife species no longer existing in the wild in Canada, but occurring elsewhere.	
Endangered (E)	A wildlife species facing imminent extirpation or extinction.	
Threatened (T)	A wildlife species likely to become endangered if limiting factors are not reversed.	
Special Concern (SC)*	A wildlife species that may become a threatened or an endangered species because of a	

combination of biological characteristics and identified threats.

Not at Risk (NAR)\*\* A wildlife species that has been evaluated and found to be not at risk of extinction given the

current circumstances.

Data Deficient (DD)\*\*\* A category that applies when the available information is insufficient (a) to resolve a species' eligibility for assessment or (b) to permit an assessment of the species' risk of

- \* Formerly described as "Vulnerable" from 1990 to 1999, or "Rare" prior to 1990.
- \*\* Formerly described as "Not In Any Category", or "No Designation Required."
- \*\*\* Formerly described as "Indeterminate" from 1994 to 1999 or "ISIBD" (insufficient scientific information on which to base a designation) prior to 1994. Definition of the (DD) category revised in 2006.

Environment Canada Canada

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Canada

The Canadian Wildlife Service, Environment Canada, provides full administrative and financial support to the COSEWIC Secretariat.

# **COSEWIC Status Report**

on the

# **Giant Threespine Stickleback**

Gasterosteus aculeatus

# **Unarmoured Threespine Stickleback**

Gasterosteus aculeatus

in Canada

2013

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Table 1.	Character means of adult Giant Threespine Stickleback from Mayer and Drizzle Lakes. Adapted from Table 6 of Reimchen <i>et al.</i> (1985). Mayer Lake data from Moodie <i>et al.</i> (1972a). Drizzle Lake data from Reimchen <i>et al.</i>

(1985). Sample sizes for Mayer Lake fish differ for each character: those indicated represent full range.

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#### WILDLIFE SPECIES DESCRIPTION AND SIGNIFICANCE

# Name and Classification

Phylum: Chordata

Class: Actinopterygii (ray-finned fishes)

Order: Gasterosteiformes

Family: Gasterosteidae

Genus: Gasterosteus

Giant Species: Gasterosteus aculeatus

Unarmoured Species: Gasterosteus aculeatus

English common name: Giant Threespine Stickleback

Unarmoured Threespine Stickleback

French common name: Épinoche à trois épines géante

Épinoche à trois épines lisse

# **Morphological Description**

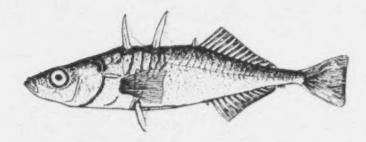
# The Giant Threespine Stickleback

The most exceptional and defining morphological feature of the Giant Threespine Stickleback is, as its name suggests, its large adult body size (Figure 1). A mean adult standard length (SL) of 75 mm has been proposed as a break between "typical" freshwater and "giant" Threespine Stickleback (Gambling and Reimchen 2012), as this exceeds the maximum size observed in the majority of lake subpopulations in Europe, Asia, and North America (Baker 1994). The two confirmed instances of Giant Threespine Stickleback clearly exceed this size limit, with mean adult body length ranging from 80.7 mm in Drizzle Lake to 84.9 mm in Mayer Lake (Table 1). Indeed, those found in Mayer Lake are the largest stickleback currently known in the circumboreal distribution of Threespine Stickleback, reaching more than 100 mm in standard length (SL; Gambling and Reimchen 2012).

Table 1. Character means of adult Giant Threespine Stickleback from Mayer and Drizzle Lakes. Adapted from Table 6 of Reimchen *et al.* (1985). Mayer Lake data from Moodie *et al.* (1972a). Drizzle Lake data from Reimchen *et al.* (1985). Sample sizes for Mayer Lake fish differ for each character: those indicated represent full range.

Character	Mayer Lake	Mayer Stream	Drizzle Lake	Drizzle Stream
Mean adult body length (mm)	84.9	50.7	80.7	49.1
Body length/pelvic spine length	5.3	6.4	6.2	6.7
Body length/body depth	4.6	4.4	5.2	4.6
Gill rakers	21.2	16.6	21.3	17.4
Lateral plates	6.8	4.7	4.9	3.6
Total vertebrae	34.0	32.5	33.3	31.8
No. of dorsal rays	11.6	10.9	12.1	11.3
No. of anal rays	9.4	8.3	9.4	8.2
Sample size	92-457	21-221	80	53

A.



B.



Figure 1. The Giant Threespine Stickleback (A, about 100 mm standard length) and Unarmoured Threespine Stickleback (B, about 65 mm standard length) drawn to the same scale. Pictures sourced from Moodie and Reimchen (1976) courtesy of T. E. Reimchen.

The Giant Threespine Stickleback is almost twice the SL of its "typical" parapatric stream counterparts (Table 1, Figure 2), and significantly longer than the mean (SL 58 mm) for Threespine Stickleback from its endemic range (Gambling and Reimchen 2012). This large and significant difference in size between a lake form and its stream counterpart is unique among known lake-stream pairs of Threespine Stickleback (Figure 3). Moreover, the defining characteristic of body size at first reproduction is a trait known to have a genetic basis (McPhail 1977).

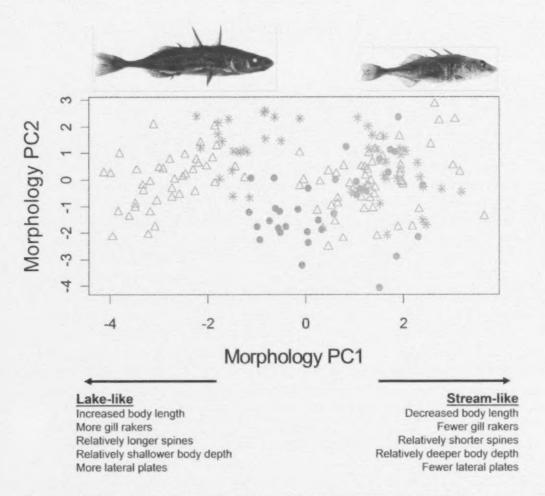


Figure 2. Morphological differentiation of Giant Threespine Stickleback (top left image and grey symbols) and their stream counterparts (top right image and orange symbols). First two principal components (PCs) from nine morphological variables illustrated (typical Mayer Lake and Gold Creek fish are shown). Symbols identify watershed: triangle = Mayer; star = Drizzle; circle = Spence. Source: Deagle et al. (2012).

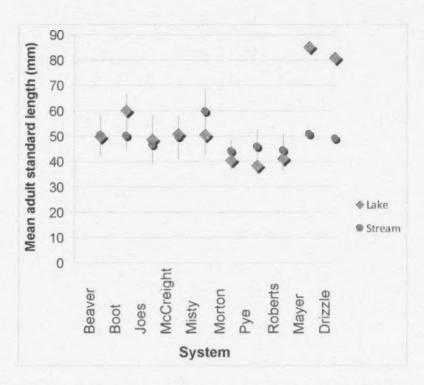


Figure 3. Mean standard length of adult lake-stream pairs of Threespine Stickleback from coastal British Columbia, Canada (standard deviation shown where data available). Non-Giant Threespine Stickleback pair system coordinates listed in Berner *et al.* (2008), data from Berner (pers. comm. 2012; lake n = 40-50; stream n = 32-51). Giant Threespine Stickleback data from Table 1.

The Giant Threespine Stickleback also diverges in several other morphometric and meristic traits from the "typical" freshwater form (Figure 2, Table 1), tending to:

- · be more streamlined
- · have more gill rakers and lateral plates
- · have longer pelvic spines
- have unusual melanistic colouration (overall black in colour with silver countershading rather than yellowish with irregular dark flank bars) with relatively drab breeding colours (Moodie 1972a; Reimchen et al. 1985).

Apart from the greatly enlarged size and more numerous lateral plates, these additional features are also shared with the other archetypal parapatric lake-stream Threespine Stickleback pair: the Misty Lake Lotic and Lentic Stickleback (Lavin and McPhail 1993; Hendry et al. 2002; Sharpe et al. 2008). Several other lake-stream pairs from Vancouver Island and Quadra Island in southwestern British Columbia that have been recently described also tend to exhibit some similar patterns of morphological divergence with these three archetypal ones, with lake fish generally being more streamlined with more gill rakers (Hendry and Taylor 2004; Berner et al. 2008, 2009), although parallelism for armour traits (pelvic and dorsal spine length, and lateral plate number) is low (Kaeuffer et al. 2012). The magnitude of divergence among these other pairs varies considerably; while some pairs show lower divergence compared to the Misty Lake pair, some are comparable, with one even exceeding this lake-stream pair (Hendry and Taylor 2004; Berner et al. 2008, Kaeuffer et al. 2012).

Experimental breeding and common garden experiments in the Misty Lake system have shown these other morphological features are inherited and have a strong genetic basis that is driven at least in part by additive genetic variation (Lavin and McPhail 1993; Hendry et al. 2002; Sharpe et al. 2008; Berner et al. 2011). Field tests investigating the phenotypic plasticity component of body shape in the Giant Threespine Stickleback from Mayer and Drizzle lakes also support a large genetic, inherited component to body shape in these fish (Spoljaric and Reimchen 2007). In two common garden experiments monitored over a decade, large bodied, highly streamlined Giant Threespine Stickleback were transplanted from their relatively large dystrophic lake (i.e., having brownish-coloured and acidic water) which has zooplankton and a full suite of vertebrate predators to smaller, shallower clearer-water eutrophic ponds with benthic prey and macroinvertebrate predators (Mayer and Drizzle ponds). The biological and physical differences between Mayer and Drizzle lakes and their respective recipient experimental ponds were ecological opposites. The results suggest that only about 10% of the total variation in body shape among Threespine Stickleback on Haida Gwaii. including the Giant Threespine Stickleback, can be attributed to plasticity (Spoljaric and Reimchen 2007).

The distinguishing suite of morphological characteristics of the Giant Threespine Stickleback, which are inherited (McPhail 1977; Lavin and McPhail 1993; Hendry *et al.* 2002; Spoljaric and Reimchen 2007; Sharpe *et al.* 2008; Berner *et al.* 2011), appear to have evolved as adaptations to divergent foraging, predation and breeding environments. Specifically:

Size:

The presence of the predatory fish Coastal Cutthroat Trout (*Oncorhynchus clarkii clarkii*) and Prickly Sculpin (*Cottus asper*) is associated with increased body size in Haida Gwaii Threespine Stickleback (Moodie and Reimchen 1976). Larger Threespine Stickleback may have a better chance of escaping predation by these gape-limited predators through sheer size, as well as potentially being better able to reach shelter when pursued (Reimchen 1988, 1991). They may also be more able to drive away sculpins that are known nest predators. Because these predators are commonly found in coastal lakes, their presence alone is not sufficient for the evolution of large body size in the Giant Threespine Stickleback. Nevertheless, the influence of predators on the maintenance of large body size in the Giant Threespine Stickleback from Mayer and Drizzle lakes is strongly supported by combined evidence from the following: Threespine Stickleback size-dependent frequencies of injuries (Reimchen 1988) and predator foraging failures (Reimchen 1991); as well as gut analyses, which have revealed low predation levels on large-bodied adult sticklebacks (Moodie 1972b; Reimchen 1991).

# Shallow body and more numerous gill rakers:

The shallower bodies of the Giant Threespine Stickleback are better suited for the sustained swimming that is typical in lakes, compared to deeper-bodied fish, which are better suited to the burst swimming and rapid maneuvering that is typical in streams (Taylor and McPhail 1986). Likewise, the more numerous gill rakers of the Giant Threespine Stickleback equip them well for feeding on the zooplankton (Bentzen and McPhail 1984; Lavin and McPhail 1986) that predominates in lakes (Hagen and Gilbertson 1972; Gross and Anderson 1984). This contrasts with fish that have fewer gill rakers, which are better suited to feeding on benthic macroinvertebrates (Bentzen and McPhail 1984; Lavin and McPhail 1986) that predominate in streams (Hagen and Gilbertson 1972; Gross and Anderson 1984). Observations of the Giant Threespine Stickleback in open lake water and their generally exposed environment agree with the interpretation that the Giant Threespine Stickleback is adapted to a pelagic life (Moodie 1972a).

# Nuptial colouration:

Although highly unusual, melanistic nuptial colouration is not unique to the Giant Threespine Stickleback, with other populations having also been identified from the northwestern Pacific (Ridgway and McPhail 1984; Scott 2001). These black forms are restricted to red-shifted habitats that are deeply tea-stained due to the presence of tannins. Here, the ambient light environment is dominated by long wavelengths compared to the clearer water usually inhabited by stickleback exhibiting the typical red colouration (Reimchen 1989; Boughman 2001; Scott 2001). The light environment likely partly explains these differences in colour; red contrasts with the background in full spectrum light whereas black contrasts with the background in red-shifted light (Reimchen 1989; McDonald et al. 1995; Boughman 2001; Scott 2001). These conspicuous colours are likely to be favored by selection because they are easier for females to see (McDonald et al. 1995; Boughman 2002). Research on benthic and limnetic species pairs of Threespine Stickleback have shown that there is both an inherited genetic basis and phenotypic plasticity underlying the correlation between male colour and light environment (Lewandowski and Boughman 2008; Malek et al. 2012).

# Robust body armour:

Research on the Giant Threespine Stickleback has demonstrated the importance of dorsal and pelvic spines, and lateral plates, as predator-defence structures (Moodie 1972b). For example, in addition to buttressing the dorsal and pelvic spines (Reimchen 1983), lateral plates may also afford protection from toothed vertebrate predators, such as Coastal Cutthroat Trout by providing integument protection from puncturing predators (Reimchen 1992a), and increasing escape opportunities (Reimchen 2000). Indeed, research strongly supports the role of predation in the evolution of the Giant Threespine Stickleback's body armour (Moodie 1972b; Reimchen 1990, 1994). For example, fish (rather than invertebrate) predation is associated with increased lateral plate numbers (Reimchen 1994).

# The Unamoured Threespine Stickleback

"Typical" freshwater Threespine Sticklebacks have three dorsal spines, an anal spine, two pelvic spines and a series of lateral bony plates. These inherited morphological structures are controlled by major genes (Colosimo *et al.* 2004, 2005; Shapiro *et al.* 2004; Chan *et al.* 2010), and are of prime importance in defence against vertebrate predators (piscivorous fish and birds; see below for adaptive interpretation).

Although most Threespine Sticklebacks develop robust defence armour, there are exceptional populations that show partial or complete loss of spines and/or lateral plates. Some of the most divergent of these Unarmoured Threespine Sticklebacks have been identified from three lakes on Haida Gwaii, and are called the Unarmoured Threespine Stickleback (Reimchen 1984). These are characterized by a loss of one or more spines in the majority of fish (Figure 4). The lateral bony plates may also be reduced or absent. Although unusual, there are other subpopulations of Threespine Stickleback across its circumboreal range that also contain a majority of fish lacking one or more spines, including elsewhere in coastal British Columbia, North America and Europe (see **Distribution** section).

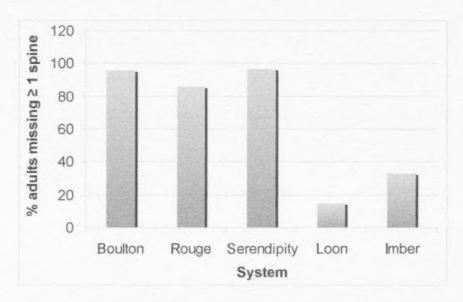


Figure 4. Proportion of adult Threespine Sticklebacks that have at least one missing spine in Haida Gwaii, British Columbia, Canada populations. Data from Moodie and Reimchen (1976); Reimchen (1980, 1984).

In Boulton Lake, the absence of the second dorsal spine and at least one pelvic spine is common (80%; Reimchen 1980). Indeed, the vast majority of fish (96%) lack at least one pelvic spine and/or the second dorsal spine, with half lacking all of these (Reimchen 1980). The absence of the first dorsal spine is rare (0.03%), with the third dorsal spine occasionally missing (2%), most often in association with the absence of the second dorsal spine (Reimchen 1980). Only 4% of fish in this lake are fully spined.

Polymorphism, including sexual dimorphism, in both the number and symmetry of pelvic spines (Moodie and Reimchen 1976; Reimchen 1980; Reimchen and Nosil 2002, 2004) and lateral plates (Reimchen and Nosil 2001c) has been identified amongst the Unarmoured Threespine Stickleback from Boulton Lake. This intrapopulation variation in defence structures is atypical and, as a result, this subpopulation has been an important subject of research study into the strength and mode of selection in natural populations, and the evolutionary causes of asymmetry (see **Special Significance** section).

Other aspects of the morphology of the Unarmoured Threespine Stickleback are unremarkable (Moodie and Reimchen 1976), with a body shape that is typical of freshwater Threespine Sticklebacks that inhabit small, shallow, stained lakes (Spoljaric and Reimchen 2007).

In Rouge Lake, the loss of the first (31%) and third (63%) dorsal spine is common, along with the loss of the anal spine (86%; Reimchen 1984). The loss of the second dorsal spine is rare (0.7%) and the pelvic spines are present (Reimchen 1984). Lateral plates are missing in 50% of fish (Reimchen 1984). Like those inhabiting Boulton Lake, the Threespine Stickleback in Rouge Lake has a body shape characteristic of those inhabiting small, shallow, stained lakes (Spoljaric and Reimchen 2007). In this instance, however, the body shape is the most derived described along this pattern of variation. It is characterized by thick caudal peduncles, posterior and closely spaced dorsal spines, anterior pelvis and small dorsal and anal fins (Spoljaric and Reimchen 2007).

In Serendipity Lake, the vast majority of fish (97%) lack pelvic spines and all fish lack lateral plates (Reimchen 1984). Dorsal spines are usually present (just 6% lack the first dorsal spine) but are reduced to vestigial projections (Reimchen 1984). Like that of the Boulton Lake sticklebacks, the body shape of the Serendipity Lake sticklebacks is typical of freshwater Threespine Sticklebacks that inhabit small, shallow, stained lakes (Spoljaric and Reimchen 2007). Their morphological resemblance to the Unarmoured Threespine Stickleback from California has been noted (Reimchen 1984).

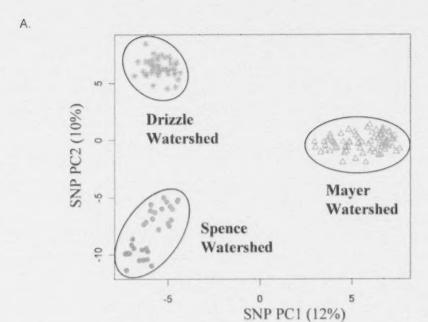
The distinguishing loss of defensive structures that characterize the Unamoured Threespine Stickleback appear to have evolved as a result of adaptation to their predation regimes; birds and macroinvertebrates are the predominant predators in these lakes that lack predatory fish (Moodie and Reimchen 1976; Reimchen 1980, 1984, 1994). Specifically, research on the Unarmoured Threespine Stickleback from Boulton Lake provides strong evidence that while the dorsal and pelvic spines are a defensive adaptation against avian predators, which are gape-limited, they are detrimental and are selected against by grappling macroinvertebrate predators, such as odonate nymphs (Reimchen 1980). Thus, intrapopulation variability in spine number appears to be a functional adaptation to spatial and temporal variability in the two predator groups, with diving birds being more prevalent in the limnetic regions in winter and odonates being most common in benthic regions in summer (Reimchen 1980; Reimchen and Nosil 2002).

# **Population Spatial Structure and Variability**

# The Giant Threespine Stickleback

The Giant Threespine Stickleback from Mayer and Drizzle lakes share a mitochondrial DNA (mtDNA) haplotype that is common and geographically widespread among northern Pacific Threespine Stickleback, and that belongs to the Eastern North Pacific lineage (O'Reilly et al. 1993; Deagle et al. 1996; Thompson et al. 1997). The high degree of mtDNA similarity among Giant Threespine Stickleback and stickleback from the surrounding marine habitat is consistent with a rapid post-Wisconsinan glacial origin of freshwater sticklebacks within the last 10,000 years (Gach and Reimchen 1989; O'Reilly et al. 1993; Thompson et al. 1997).

The question remains as to whether the Giant Threespine Stickleback has evolved in parallel multiple times under similar selection regimes or rather has a single origin. Freshwater stickleback inhabiting different coastal watersheds are generally considered to be independently derived from the marine forms (Hagen and McPhail 1970; McPhail and Lindsey 1970; Bell 1976). Following from this, the convergence in morphology of the different Giant Threespine Stickleback in Mayer and Drizzle lakes has been assumed to be independently derived, even though they are less than 50 km apart (Reimchen et al. 1985). Genetic investigations support the hypothesis that the Giant Threespine Stickleback of Mayer and Drizzle lakes evolved independently from one another by parallel evolution; mtDNA (Thompson et al. 1997) and genome-wide single nucleotide polymorphism (SNP) analyses (Figure 5) show that, rather than clustering together, the Giant Threespine Stickleback clusters with its inlet counterparts within each watershed. This is reinforced by the fact that the majority of genomic outlier regions identified through the population genomics approach were watershed-specific (Deagle et al. 2012).



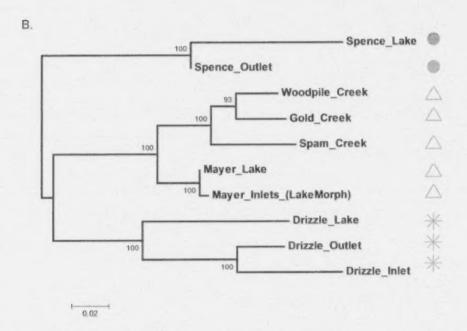


Figure 5. Genetic differentiation of Giant Threespine Stickleback (grey) and their stream counterparts (orange). A. First two principal components (PCs) from 760 SNPs (evenly distributed, non-sexed linked loci) illustrated. B. Population-level neighbour-joining tree based on F<sub>ST</sub> across the 760 SNP loci. Per cent bootstrap support (1,000 replicates) shown at nodes. Source: Deagle *et al.* (2012).

Mitochondrial DNA analysis also strongly supports the independent origin of the Giant Threespine Stickleback from the other archetypal parapatric lake-stream Threespine Stickleback pair in British Columbia, the Misty Lake Lotic and Lentic Stickleback (Thompson et al. 1997); not only did the Giant Threespine Stickleback not cluster with the Misty Lake Threespine Stickleback, fish in the two lakes did not even share any mtDNA haplotypes (Thompson et al. 1997). The separate evolution of the Giant Threespine Stickleback and its stream counterparts is consistent with genetic data from other Threespine Stickleback species pairs from coastal British Columbia (stream-lake stickleback: Berner et al. 2009; benthic-limnetic stickleback: Taylor and McPhail 2000).

A further question remains as to whether each Giant Threespine Stickleback subpopulation and their respective stream forms are the result of a single colonization with secondary modifications (parapatric divergence) or multiple, independent divergence events with secondary contact (allopatric divergence). Reimchen et al. (1985) argued that it is reasonable to suppose parapatric divergence occurred in response to different selection pressures on opposite sides of an ecotone through two sequential, post-Wisconsinan speciation events: an initial evolution of a stream form near the outlet mouth led to upstream dispersal into the lake and inlets; Giant Threespine Stickleback in the lake was secondarily derived from this stream form, while all inlet subpopulations represent a genetic continuum of the same widely distributed phenotype (Reimchen et al. 1985).

It is difficult to tease apart allopatric and parapatric origins for recently derived species such as the Giant Threespine Stickleback and its inlet counterparts (Endler 1982). Nevertheless, the clustering of the Giant Threespine Stickleback with its stream counterparts within each watershed, as revealed by mtDNA and SNP analyses, support the plausibility of a postglacial ecological parapatric divergence along the lake-stream ecotone (Thompson *et al.* 1997; Figure 5). In particular, that stream form fish within each watershed are generally more genetically similar to one another than they are to the Giant Threespine Stickleback (Figure 5) supports the idea of a genetic continuum of the stream form distinct from the Giant Threespine Stickleback.

A genome-wide genetic analysis using SNP genotyping reveals significant differentiation between the Giant Threespine Stickleback and its stream counterparts within both Drizzle and Mayer watersheds;  $F_{\rm ST}$  [a measure of genetic divergence between populations ranging from 0 to 1.0] was estimated at 0.14 and 0.17 between Drizzle Lake and its outlet and inlet, respectively, when SNPs subject to natural selection (so-called "outlier" loci) were removed from analysis; and  $F_{\rm ST}$  ranged from 0.06 to 0.08 between Mayer Lake and its three inlets (Deagle *et al.* 2012). These  $F_{\rm ST}$  values are within the range of those reported from smaller population genetic surveys of other parapatric lake-stream pairs of Threespine Stickleback from coastal British Columbia that are thought to have evolved at least in part through ecological divergence since the last glaciation (Hendry *et al.* 2002; Berner *et al.* 2009). Their differentiation suggests that the Giant Threespine Stickleback are at least partially reproductively isolated in parapatry from their stream counterparts. The Giant Threespine Stickleback is also clearly genetically differentiated from marine Threespine Stickleback from the Pacific Ocean (Jones *et al.* 2012).

# The Unarmoured Threespine Stickleback

Populations of Threespine Stickleback with reduced armature, including the Unarmoured Threespine Stickleback, are most likely independently derived from the marine ancestor (Bell 1987). This has allowed recent studies into the repeated evolution of pelvic reduction (e.g., Chan et al. 2010). Unlike the Giant Threespine Stickleback, the Unarmoured Threespine Stickleback from Boulton, Rouge and Serendipity lakes do not belong to a single mtDNA lineage. While Boulton Lake contains fish solely belonging to the Euro-North American lineage (sensu Johnson and Taylor 2004), Threespine Stickleback from Rouge Lake belong to the Trans-North Pacific lineage (O'Reilly et al. 1993; Deagle et al. 1996; Thompson et al. 1997; Johnson and Taylor 2004). Serendipity Lake, on the other hand, contains fish that belong to both lineages (two thirds Trans-North Pacific lineage, N = 12; O'Reilly et al. 1993; Thompson et al. 1997; Deagle et al. 2012). That armour reduction is found in fish belonging to both lineages strongly supports the independent origin of these subpopulations even within this small geographic area. This is reinforced by divergent developmental patterns of pelvic reduction in Boulton and Serendipity lakes (Bell 1987). That is, they appear to have evolved their derived unarmoured morphology in parallel multiple times.

Recent genome-wide SNP analyses (Jones et al. 2012) further supports the hypothesis that the Unarmoured Threespine Stickleback from Boulton and Rouge lakes are genetically and evolutionarily distinct from one another (Serendipity Lake was not included in the analysis). This study showed that the two populations are genetically independent, being more similar to other subpopulations of freshwater Threespine Stickleback than they are to one another (Jones et al. 2012). They are also clearly genetically differentiated from Pacific Ocean Threespine Stickleback (Jones et al. 2012).

The geographical location and radiocarbon-dating of these lakes (reviewed in Deagle et al. 2012), along with their high degree of mtDNA similarity to neighbouring freshwater subpopulations containing fish from their respective mtDNA lineages, strongly support a rapid post-Wisconsinan glacial origin of each of these populations following colonization of freshwater habitats within the last 10,000 years (Gach and Reimchen 1989; O'Reilly et al. 1993; Deagle et al. 2012).

# **Designatable Units**

The Giant and Unarmoured Threespine Sticklebacks each warrant separate designatable unit status within *Gasterosteus aculeatus* because they satisfy the "discrete" and evolutionary "significant" criteria of COSEWIC (COSEWIC 2011).

# The Giant Threespine Stickleback

The Giant Threespine Stickleback is discrete from other Threespine Stickleback; an assemblage of inherited traits (morphological and behavioural) and genetic data (mtDNA and SNP) support the view that it is genetically distinct from other Threespine Sticklebacks, including their "typical" parapatric stream counterparts:

- The morphological differences between the Giant Threespine Stickleback and its parapatric stream forms (Figure 2, Deagle et al. 2012) have a strong genetic basis that is driven at least in part by additive genetic variation (Lavin and McPhail 1993; Hendry et al. 2002; Spoljaric and Reimchen 2007).
- Morphological and genetic analyses suggest that there is very little hybridization between the Giant Threespine Stickleback and its parapatric stream forms (Moodie 1972a; Reimchen et al. 1985; Gach and Reimchen 1989; Deagle et al. 2012).
- Population genetic analysis reveals genetic differentiation between the Giant Threespine Stickleback and its stream counterparts (Figure 5; Deagle et al. 2012) suggesting at least partial reproductive isolation within both Mayer and Drizzle watersheds.
- This is reinforced by mate preference tests that provide evidence of positive assortative mating between the Giant Threespine Stickleback and its stream counterparts (Moodie 1972a; Stinson 1983).

The Giant Threespine Stickleback is evolutionary significant: it represents the most extreme cases of gigantism known among lake-stream pairs of Threespine Stickleback (Figure 3) and, indeed, in *G. aculeatus* despite the sampling of hundreds of coastal lakes (see **Distribution** section). Loss of these discrete populations would, therefore, restrict the range of morphological variability in *G. aculeatus* as a whole.

The morphological character complex that sets them apart from other Threespine Stickleback (Figure 2) is composed of a suite of inherited traits, the persistence of which is the result of evolutionary divergences. These divergent populations exist within an ecological and evolutionary setting in which their associated adaptations (foraging, predation and breeding) are crucial to their persistence in parapatry with "typical" stream forms (see above discussion of adapation and **Morphological Description** section).

# The Unarmoured Threespine Stickleback

The Unarmoured Threespine Stickleback is discrete from other populations of Threespine Stickleback; an assemblage of inherited traits and genetic data support the view that it is genetically distinct from other Threespine Sticklebacks:

- The morphological trait that distinguishes this fish (spine loss) from other populations
  of Threespine Stickleback on Haida Gwaii and from across most of the global range
  of G. aculeatus (Figure 4; Reimchen 1984) is controlled by major genes (Shapiro et
  al. 2004; Chan et al. 2010).
- Genetic analyses (mtDNA and SNP) demonstrate that it is genetically distinct from other Threespine Stickleback (O'Reilly et al. 1993; Deagle et al. 2012; Thompson et al. 1997; Jones et al. 2012).

The Unarmoured Threespine Stickleback in Boulton, Rouge and Serendipity Lakes are geographically isolated from each other and all other *G. aculeatus*. Boulton Lake has no inflow streams, and is maintained with groundwater seepage. Its outflow drainage is intermittent (Reimchen 1984) and is too steep to support Threespine Stickleback (Gach and Reimchen 1989). Both Rouge and Serendipity lakes are considered to be closed systems; like Boulton Lake, they have no inlets and are maintained by groundwater seepage, and beaver dams prevent immigration into the lakes via their outlets (Reimchen 1984; Deagle *et al.* 1996). Emigration out of these lakes is possible, however, and downstream gene flow is congruent with the molecular and morphological cline that has been described from Rouge Lake (fish are unarmoured and monomorphic for the Trans-North Pacific lineage) to the mouth of its outlet (armoured and are monomorphic for the Euro-North American lineage; Deagle *et al.* 1996).

The Unarmoured Threespine Stickleback is evolutionary significant: its populations represent some of the most extreme cases of Unarmoured Threespine Sticklebacks that have been described among the many hundreds of populations that have been sampled across the global range of *G. aculeatus* (see **Distribution** section). Loss of these discrete populations would, therefore, eliminate a significant aspect of the morphological diversity of *G. aculeatus* as a whole.

The persistence of the loss of defensive structures that characterize the Unamoured Threespine Stickleback is the result of evolutionary divergences. These divergent populations exist within an ecological and evolutionary setting in which their associated adaptations to divergent predation regimes are crucial to their persistence (see above discussion of adaptation and **Morphological Description** section).

# **Special Significance**

The significance of the Giant and Unarmoured Threespine Sticklebacks is primarily their contribution to Canada's biodiversity and their scientific value. They also have intrinsic value as significant prey items in their ecosystems (see **Interspecific Interactions** section).

The Giant Threespine Stickleback is highly endemic, with a known global range of just two lakes. The three lakes harbouring the Unarmoured Threespine Stickleback represent a significant proportion of the Canadian and global range of Unarmoured Threespine Stickleback (loss of one or more spines in the majority of fish). Both contribute to the extensive morphological variation displayed by Threespine Stickleback from Haida Gwaii. This endemic radiation is at least as broad as the spectrum of variation evident across the rest of the circumboreal distribution of Threespine Stickleback (Moodie and Reimchen 1976; Spoljaric and Reimchen 2007). More broadly, they are part of a highly endemic assemblage of native biodiversity that characterizes Haida Gwaii (reviewed in Moodie and Reimchen 1973).

The populations of the Giant Threespine Stickleback constitute the lake form of two of the three archetypal parapatric lake-stream Threespine Stickleback pairs (Lavin and McPhail 1993; Hendry et al. 2002). Other lake-stream pairs have more recently been described from Vancouver Island and Quadra Island in southwestern British Columbia (Hendry and Taylor 2004; Berner et al. 2008, 2009; Kaeuffer et al. 2012). All of these divergent pairs share some morphological characteristics, and have evolved separately through parallel evolution (see **Population Spatial Structure and Variability** section).

Both the Giant and Unarmoured Threespine Sticklebacks have yielded significant insights to the study of evolutionary processes. Their distinct morphological characteristics provide rare opportunities to investigate the ecological and evolutionary causes of morphological variance. Specifically, both the Giant and the Unarmoured Threespine Stickleback have given insight into:

 Abiotic and biotic factors driving the evolution of body shape (Spoljaric and Reimchen 2007, 2011).

- Associations between fitness and asymmetry (Bergstrom and Reimchen 2000, 2002), including the role of ecological selection (e.g. divergent microhabitat, diets, predators), in driving differences in asymmetry of predator defence structures (pelvic-girdle and lateral plates) and parasitism (Reimchen 1997; Reimchen and Nosil 2001a,b,c; Bergstrom and Reimchen 2003, 2005; Reimchen and Bergstrom 2009). This work is a significant contribution to literature on the evolutionary implications of developmental instability and intrapopulation variability.
- The evolution of sexual dimorphism (Reimchen and Nosil 2004; Spoljaric and Reimchen 2008).
- They are now also contributing to pioneering genomic studies that are exploring the genetic basis of adaptation (Chan et al. 2010; Jones et al. 2012).

In addition, the Giant Threespine Stickleback has given insight into selection from predation regime on body size and armour (see **Morphological Description** section), while the Unarmoured Threespine Stickleback has been useful in studies examining spatial and temporal variation in ecological selection (from divergent predation regime) on armour (Reimchen 1980; Reimchen and Nosil 2002).

#### DISTRIBUTION

# **Global Range**

# The Giant Threespine Stickleback

The known range of the Giant Threespine Stickleback is highly restricted, being endemic to Haida Gwaii on the west coast of British Columbia (Figure 6). It occurs only in the Pacific Islands National Freshwater Biogeographic Zone, having been confirmed from just two lakes: Mayer Lake in the Mayer River drainage and Drizzle Lake in the Sangan River drainage, both located in the northeast of Graham Island, the most northerly island of Haida Gwaii (Moodie 1972a, 1984; Moodie and Reimchen 1973, 1976; Reimchen 1984; Reimchen et al. 1985). The distribution of these extant native populations has not changed since their discovery several decades ago. It is, however, possible that other populations of Giant Threespine Stickleback may yet be described (see **Search Effort** section).

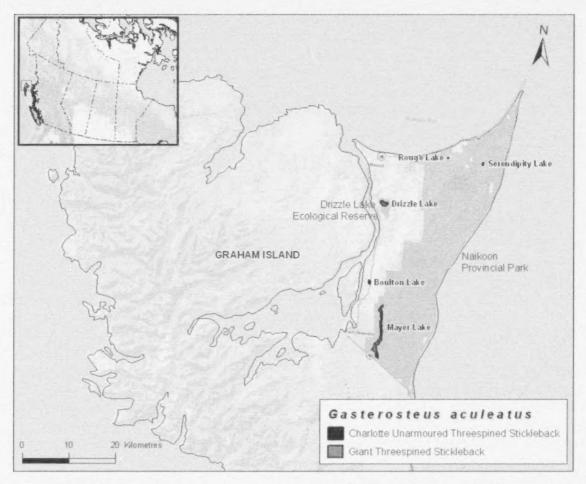


Figure 6. Distribution of native populations of the Giant and Unarmoured Threespine Sticklebacks in Canada. Current and historical distributions are identical, as are global and Canadian ranges. Data from Moodie (1984); Riemchen (1984); Reimchen et al. (1985).

# The Unamoured Threespine Stickleback

The Unarmoured Threespine Stickleback is part of a larger circumboreal complex of Threespine Stickleback subpopulations that exhibit a loss of one or more spines in the majority of their fish. The Unarmoured Threespine Stickleback refers to the populations found on Haida Gwaii on the west coast of British Columbia (Figure 6). It occurs only in the Pacific Islands National Freshwater Biogeographic Zone. It is known to occur in at least three lakes in separate drainages on Graham Island: Boulton, Rouge, and Serendipity lakes (Moodie and Reimchen 1976; Reimchen 1980, 1984). The distribution of these extant native populations has not changed since their discovery several decades ago, although it is possible that other populations of the Unarmoured Threespine Stickleback may yet be described (see **Search Effort** section).

## **Canadian Range**

Since both the Giant Threespine Stickleback and the Unarmoured Threespine Stickleback are endemic to Canada, the Canadian and global ranges for each of these species are identical (Figure 6).

## **Extent of Occurrence and Area of Occupancy**

The extent of occurrence (EO) and index of area of occupancy (IAO) were estimated for the Giant and Unarmoured Threespine Sticklebacks according to the COSEWIC guidelines (i.e. using the minimum convex polygon method for EO, and using an overlaid grid of 2 km x 2 km cells for IAO). Based on its confirmed distribution, the EO for the Giant Threespine Stickleback is estimated to be 63 km². The biological area of occupancy is approximately 739 ha, and the IAO is 52 km² calculated from thirteen 2 km x 2 km cells. The EO for the Unarmoured Threespine Stickleback is estimated to be 124 km² based on its confirmed distribution. The biological area of occupancy is 22 ha, and the IAO is 20 km² calculated using five 2 km x 2 km cells.

The most likely or imminent threat to the Giant Threespine Stickleback comes from the introduction of invasive species. A decline in predation pressure from Coastal Cutthroat Trout (e.g., from overfishing) and/or Common Loon (e.g., from recreational disturbance) is likely also a significant threat (see **Threats and Limiting Factors** section below). The probable extent of this threat is the entire lake for each of the two lakes occupied by this species and they would act independently across each lake. Two locations (Mayer and Drizzle lakes) are, therefore, recognized. For the Unarmoured Threespine Stickleback, the most likely or imminent threats come from the introduction of invasive species, particularly the introduction of gape-limited predators (e.g., Coastal Cutthroat Trout; see **Threats and Limiting Factors**). The probable extent of this threat is the entire lake for each of the three lakes occupied by this species and would act independently across lakes. Three locations (Boulton, Rouge and Serendipity lakes) are, therefore, recognized.

#### Search Effort

Many hundreds of lakes along the British Columbia, Washington and Alaska coasts have been surveyed for Threespine Stickleback, and many more throughout their global range (e.g., Bell and Foster 1994). Extensive surveys of lakes in Haida Gwaii (Moodie and Reimchen 1976; Reimchen et al. 1985; Reimchen 1989, 1994; Spoljaric and Reimchen 2007) have identified the Giant and Unarmoured Threespine Sticklebacks from only a handful of lakes on Graham Island, Haida Gwaii, British Columbia (Moodie 1972a, 1973, 1984; Moodie and Reimchen 1976; Reimchen 1980, 1984; Reimchen et al. 1985). Given the inaccessible nature of this area, however, it is possible that there may be more instances of the Giant and Unarmoured Threespine Sticklebacks yet to be described.

# The Giant Threespine Stickleback

Although Giant Threespine Sticklebacks have been well described from only two lakes (Moodie 1972a, 1973, 1984; Moodie and Reimchen 1976; Reimchen 1984; Reimchen et al. 1985), a recent report suggests there are other "large-bodied" Threespine Stickleback (SL >75 mm): a survey of 140 allopatric lakes on Haida Gwaii found "large-bodied" Threespine Stickleback specimens in six other lakes, each in a separate watershed: Skidegate (maximum SL 94), Laurel (90), Eden (87), Escarpment (87), Coates (81), and Spence (77) compared to Drizzle (96) and Mayer (106; Gambling and Reimchen 2012). In these other instances, however, accessible morphological data sets from these lakes (Moodie and Reimchen 1976; Spoljaric and Reimchen 2007; Gambling and Reimchen 2012) are currently inadequate to calculate mean adult body length. Consequently, the status of sticklebacks from these lakes on Haida Gwaii and other lakes in adjacent areas (e.g., ponds and lakes on the Banks-Estevan archipelago, 120 km to the east of Haida Gwaii; Reimchen and Nosil 2006) as Giant Threespine Stickleback must await availability of the data required to evaluate their average size and other characteristics.

## The Unarmoured Threespine Stickleback

In addition to the Unarmoured Threespine Stickleback, there are other populations of Threespine Stickleback across its circumboreal range that contain a majority of fish lacking one or more spines. A recent survey of seventy ponds and lakes from the Banks-Estevan archipelago, 120 km to the east of Haida Gwaii, found a population, Barnard Island SAMPLE B55, where 62% of the fish had spine loss, primarily of the first dorsal spine (Reimchen and Nosil 2006). So Threespine Sticklebacks with reduced armour are not endemic to Haida Gwaii in this region. Other subpopulations of Threespine Stickleback that have a majority of individuals displaying partial or complete loss of the pelvic skeleton include populations from southern coastal British Columbia (McPhail 1993; Gow et al. 2008), Québec (Edge and Coad 1983), Alaska (Bell and Ortí 1994), the Outer Hebrides, Scotland (Campbell 1979, 1984; Bell 1987), and Iceland (Shapiro et al. 2004).

## HABITAT

# **Habitat Requirements**

Earlier work suggested a relatively close concordance between lake habitat and ecosystem with stickleback morphology on Haida Gwaii (Reimchen 1994). This showed that the Unarmoured Threespine Stickleback was restricted to shallow bog ponds with no predatory fish and few predatory birds, whereas the Giant Threespine Stickleback was restricted to larger dystrophic lakes with predatory fish and avian piscivores (Reimchen 1994). More recent discoveries, however, describe the occurrence of other "large-bodied" Threespine Stickleback in a diversity of lake habitats that range from relatively large, clear oligotrophic mountain lakes through to a smaller, shallow dystrophic pond (Reimchen and Nosil 2006; Gambling and Reimchen 2012). The importance of lake habitat features to the persistence of the Giant and Unarmoured Threespine Stickleback awaits further investigation.

Habitat requirements for both of these species most likely include those same features that may limit the size or viability of other lake subpopulations of Threespine Stickleback (e.g., nesting habitat area, juvenile rearing area). These needs most probably include sustained littoral and pelagic productivity, absence of invasive species, and maintenance of gently sloping sand/gravel beaches and natural littoral macrophytes for nesting and juvenile rearing.

# The Giant Threespine Stickleback

Two dystrophic lakes on Graham Island, Haida Gwaii of British Columbia, Canada hold Giant Threespine Stickleback (Moodie and Reimchen 1973, 1976). Both lakes are located within the Queen Charlotte Lowland Ecoregion (Figure 6), poorly drained lowlands that are dominated by *Sphagnum* bogs and coniferous forest. In both instances, the Giant Threespine Stickleback is confined to its lake habitat, and does not enter connecting streams (see **Dispersal and Migration** section).

Mayer Lake is an open lake, with three tributary inlet streams (Cott, Woodpile, Gold creeks), and flows into the Pacific Ocean through the Mayer River (Moodie 1972a). It is 22 m above sea level, 12 km long, averages 0.8 km in width, and has a maximum depth of 10 m (Moodie 1972a). It has a surface area of about 627 ha (Moodie and Reimchen 1976) and is characterized by low calcium levels (<1 ppm) and pH (5.5; Moodie 1972a), with waters that are heavily tannin-stained (57 % transmission at 400 nm [T<sub>400</sub>]; Reimchen 1989). Much of the littoral habitat is gentle slopes of sand or pebbles, with scattered patches of vegetation, predominantly *Nuphar*, water lily, *Fontinalis*, water moss, and *Isoetes*, reed (Moodie 1972a). In contrast, the inlet streams tend to be muddy and densely covered with mats of *Sphagnum* moss and stands of emergent grasses (Moodie 1972a). The inlet mouths are a transition zone where lake and stream habitat characteristics merge (Moodie 1972a).

Drizzle Lake occurs on the Argonaut Plain (< 100 m elevation) in the northeast corner of the Queen Charlotte Lowland Ecoregion. It is an open lake that is part of the Sangan River watershed; it has one inlet and an outlet that connects it to the Pacific Ocean (Reimchen *et al.* 1985). It has a surface area of 112 ha and is shallow (max depth < 30 m, Reimchen 1994). The lake bottom consists mostly of sand and gravel. Some pebble beaches occur around the shoreline (BC Parks 2012). It is characterized by low calcium levels (<1 ppm), low pH (4.0-5.5; Reimchen *et al.* 1985), and heavily tannin-stained waters ( $T_{400}$  = 67 %; Reimchen 1989). Aquatic vegetation is sparse: localized stands of *Nuphar*, *Sparganium*, and *Juncus* (Reimchen *et al.* 1985).

The Giant Threespine Stickleback is adapted to a generally pelagic life although they prefer to nest in the shallower littoral zone of these lakes in vegetation stands on gently sloping sandy substrate (Moodie 1972a, 1984). As a result, they tend to spend spring and summer near the shore to spawn but overwinter in deeper water (Moodie 1972a, 1984).

# The Unarmoured Threespine Stickleback

Three small (< 20 ha) lakes on Graham Island, Haida Gwaii of British Columbia, Canada hold the Unarmoured Threespine Stickleback (Moodie and Reimchen 1973, 1976; Reimchen 1984). These lakes all occur on the Queen Charlotte Lowland Ecoregion, which covers the forested plain and wetland complex of northern and eastern Graham Island (Figure 6). This region is characterized by wetlands in association with coniferous forests, and each of these lakes is surrounded by *Sphagnum* bog and scrub coniferous forest (Reimchen 1984).

Boulton Lake is a small (18 ha), shallow ( $< 5 \, \text{m}$ ), acidic (pH 4.7) lake (Reimchen 1984). It is fed principally by groundwater seepage, and has an intermittent outlet draining to marine waters that are several kilometres distant (Moodie and Reimchen 1976, Reimchen 1980, 1984). It is 60 m above sea level (Reimchen 1980). The lake substrate is thick organic ooze in the southern half, and sand and gravel in the central and northern sections (Reimchen 1980, 1984). Floating and submerged vegetation is common, and includes about 10 % cover by *Nuphar* and locally abundant *Scirpus* (Reimchen 1980, 1984). Its water is clearer ( $T_{400} = 77 \, \%$ ) than the relatively stained waters of Rouge ( $T_{400} = 68 \, \%$ ) and Serendipity lakes ( $T_{400} = 71 \, \%$ ; Reimchen 1989). Habitat differences between the sexes have been described for Boulton Lake; with adult females being primarily limnetic in spring and summer, and adult males remaining nearer shore to nest (Reimchen 1980).

Rouge Lake is smaller (2 ha), shallower (< 2m) and more acidic (pH 4.1-4.5) than Boulton Lake (Reimchen 1984). It is a closed lake that is fed principally by groundwater seepage, with a beaver dam blocking the outflow (Reimchen 1984). The lake substrate is thick organic ooze and sand (Reimchen 1984). *Sphagnum* overhangs the shoreline, and *Nuphar* covers about 50% of the lake (Reimchen 1980, 1984).

Serendipity Lake is similar in many regards to Rouge Lake: it is similar in size (2 ha), depth (< 2 m) and acidity (pH 3.9-4.3; Reimchen 1984). It is also closed, being fed primarily by groundwater seepage, with its outflow blocked by a beaver dam (Reimchen 1984). The lake substrate is thick organic ooze, *Sphagnum* overhangs the shoreline, and *Nuphar* covers about 50% of the lake (Reimchen 1984).

The loss of armour in these fish is probably tightly linked to the small size and acidic conditions of these lakes; these attributes exclude fish and large avian predators (Reimchen 1984).

### **Habitat Trends**

Trends in habitat quantity and quality can be assessed only qualitatively because there has been no long-term monitoring of the habitat of the Giant or the Unarmoured Threespine Stickleback.

## The Giant Threespine Stickleback

Drizzle and Mayer lakes are natural ecosystems that do not appear to have undergone any significant habitat changes in recent times (Moodie 1984; Reimchen 1994). Their habitat may be considered stable, although it should be noted that there is no information available on the rate of habitat change over the last decade.

# The Unarmoured Threespine Stickleback

The natural drainage of the Queen Charlotte Lowland Ecoregion changed in the latter half of the twentieth century after the introduction of the North American Beaver (*Castor canadensis*) by the British Columbia Game Commission (Reimchen 1984). Small lakes (<20 ha) were most adversely affected by rising water levels, with loss of sandy littoral areas in several lakes and a general increase in surface area of others (Reimchen 1984). Some previously isolated lakes became connected (Reimchen 1984).

Boulton Lake was not reported to have undergone significant changes as a result of beaver activity (Reimchen 1984) but there is no information available on the rate of any habitat change over the last decade. Rouge and Serendipity Lakes both had their outlet streams blocked by beaver activity, the former in the early 1970s, and the latter at the end of the same decade (Reimchen 1984). As a result, lake levels rose up to 1 m. In Rouge Lake, this eliminated most of the shallow sandy littoral beaches, with those remaining largely covered in dead organic debris (Reimchen 1984). These changes may have impacted reproduction (potentially disrupting mate recognition and nesting habitat) and interspecific interactions (potentially increasing lake use by predatory birds such as loons). Since that time, however, the water levels appear to have stabilized (Reimchen 1984) although there is no information available on the rate of any habitat change over the last decade.

### **BIOLOGY**

# Life Cycle and Reproduction

## The Giant Threespine Stickleback

The reproductive biology of the Giant Threespine Stickleback described from Mayer Lake appears to be largely similar to other freshwater Threespine Stickleback (Östlund-Nilsson 2006). The breeding season lasts about 90 days beginning in early May, peaking in June with few breeding fish by mid-August (Moodie 1972a). This pattern is probably similar to the stream form, and is typical of Threespine Stickleback subpopulations at this latitude (Moodie 1972a). Within the breeding season, the territorial male Giant Threespine Stickleback probably completes up to five breeding cycles, with each cycle lasting about 18 days: 1 day to build the nest and court females, 9 days of egg incubation, and 8 days care of the fry (Moodie 1972a, 1984). In each breeding cycle, male sticklebacks mate with approximately three females (Moodie 1972a). They nest in clumps where the substrate is sand or gravel with some shelter e.g., from Fontinalis or rocks (Moodie 1984). Those nesting close to shelter appear to be more successful in rearing their offspring to an advanced stage than are males which nest farther from shelter (Moodie 1972a).

The Giant Threespine Stickleback does, however, have several striking deviations in reproductive biology from most other populations of Threespine Stickleback. First is the loss of male red nuptial colouration (see adaptive interpretation of distinguishing morphological characteristics in **Morphological Description** section; Reimchen 1989).

Second, fecundity in *Gasterosteus* is closely correlated with standard length (Hagen 1967). It is, therefore, no surprise that the Giant Threespine Stickleback produces more than twice as many eggs as a wild stream form (mean = 257 eggs per clutch in Mayer Lake [Moodie 1972a, 1984]; mean = 395 eggs per clutch in Drizzle Lake [Reimchen 1990]). These eggs are also considerably larger than those of other wild subpopulations (Moodie 1972a).

Third, Threespine Sticklebacks are typically short-lived, reaching reproductive age in their second or third summer, at approximately 12 or 24 months (Wootton 1976) and dying shortly after one or two breeding cycles. Its maximum life span is typically 2 or 3 years but occasionally reaches 4 years (Pennycuick 1971; Moodie 1984; Baker 1994). Remarkably, "large-bodied" Threespine Sticklebacks of Haida Gwaii (including the Giant Threespine Stickleback) reach maximum life spans that range from at least 4 to 8 years old (4 years old for Mayer Lake and 8 years old for Drizzle Lake; Reimchen 1992b; Gambling and Reimchen 2012). Breeding males appear to be at least two years old (third summer) at first reproduction in Mayer Lake (Moodie 1972a, 1984), and at least three years old in Drizzle Lake (Reimchen 1992b) but appear to remain reproductive throughout their extended life (Reimchen 1992b; Gambling and Reimchen 2012). The maximum life spans within this group of "large-bodied" Threespine Stickleback does not significantly correlate with body size, however, reflecting large differences in growth rate (Gambling and Reimchen 2012). Factors contributing to these occurrences of exceptional lifespan remain speculative although low productivity habitats and refuge against gape-limited piscivores, each of which theoretically predicts reduced rate of senescence, are associated with the greatest longevity among these populations (Gambling and Reimchen 2012).

## The Unarmoured Threespine Stickleback

Although it has been noted that the Unarmoured Threespine Stickleback has a breeding structure similar to other *G. aculeatus* (Reimchen 1984), little of the reproductive biology of the Unarmoured Threespine Sticklebacks has been described. They reach sexual maturity in their third year, with females producing 100-300 eggs per clutch (Reimchen 1984). They are likely largely similar to other populations of Threespine Stickleback (Östlund-Nilsson 2006).

# Physiology and Adaptability

The Giant Threespine Stickleback is tolerant of low calcium levels (<1 ppm), low pH (4.0-5.5), and heavily tannin-stained waters (57-67 % transmission at 400 nm  $[T_{400}]$ ) that are found in the dystrophic lakes it inhabits (see **Habitat Requirements** section).

The Unarmoured Threespine Stickleback is also tolerant of acidic waters. The original status report on this fish did, in fact, report that the tolerance of the fish found in Serendipity Lake to acidic waters was unparalleled for fish survival (Reimchen 1984).

In general, Threespine Sticklebacks are sensitive to stress in the environment and are good bioindicators in ecotoxicological research (e.g., Scholz and Mayer 2008). Nevertheless, *G. aculeatus* adapt readily to change, including anthropogenic disturbance (reviewed in Candolin 2009). Non-intuitively, this adaptability may be an underlying vulnerability for the Giant and Unarmoured Threespine Sticklebacks. Each has evolved in response to specific selective forces (most likely including specific habitat conditions and predator regimes; see **Habitat Requirements** and **Interspecific Interactions** sections). Changes in the selective regimes could lead to adaptive alterations in phenotype that would result in loss of their morphological distinctness.

Threespine Sticklebacks are easily artificially reared, and these two species would likely survive transplantation (either as artificially reared or wild fish) to lakes that had similar physical and chemical characteristics. Indeed, wild fish transplanted from Mayer and Drizzle lakes to nearby ponds as part of two common garden experiments have persisted over decades (Spoljaric and Reimchen 2007). However, the maintenance of their distinct phenotypes and their genetic integrity would most likely depend on similar selective pressures, including predator regime as well as the physical and chemical attributes of the lakes. Indeed, differences in body shape were detected between source and transplanted subpopulations in ecologically opposite habitats after just one generation (Spoljaric and Reimchen 2007). Even if these fish were transplanted to superficially similar lakes, the success of transplanting can in no way be assured given that:

- Other lakes close by to those in which the species are found are at least superficially similar do not support these species but harbour more "typical" freshwater forms of Threespine Stickleback (Moodie and Remchen 1976; Reimchen 1984; Reimchen et al. 1985).
- Our understanding of the specific lake habitat features that are essential to the persistence of the Giant and Unarmoured Threespine Stickleback is incomplete (see Habitat Requirements section).

## **Dispersal and Migration**

## The Giant Threespine Stickleback

Drizzle and Mayer lakes are connected to marine waters via their outlets (Moodie 1972a; Reimchen et al. 1985) so migration of the Giant Threespine Stickleback to marine waters, as well as gene flow with stream forms, can potentially occur. Nevertheless, the Giant Threespine Stickleback is largely confined to its lakes, spending spring and summer near the shore to spawn and overwintering in deeper water (Moodie 1972a). Extensive sampling has not found any Giant Threespine Stickleback in the outlet streams (Moodie 1972a; Reimchen et al. 1985; Deagle et al. 2012), with only recent sampling recovering some from Mayer Lake inlets (Deagle et al. 2012). Similarly, stream form fish have only been detected in the lake near the stream mouths in Mayer Lake (Mayer 1972a). While these fish can presumably have ecological interactions when they do occasionally occur in sympatry, morphometric and genetic analyses

reveal no indication of introgression or hybridization; there is no clinal morphological variation along the streams nor a trend towards morphological intermediacy (Moodie 1972a; Reimchen *et al.* 1985; Gach and Reimchen 1989; Deagle *et al.* 2012), with lake and stream forms being genetically distinct at an inlet's zone of sympatry (Deagle *et al.* 2012).

# The Unarmoured Threespine Stickleback

The Unarmoured Threespine Stickleback in Boulton, Rouge and Serendipity lakes are geographically isolated from one another and from other *G. aculeatus*. Within each lake, adult males and fry spend spring and summer near the shore to spawn and rear, and overwinter in deeper water (Reimchen 1984). Fernales and subadults are found in open water close to the surface during the warmer months of summer, and in the benthic regions during winter (Reimchen 1980, 1984).

Boulton Lake has no inflow stream, and is maintained with groundwater seepage. Its outflow drainage is intermittent (Reimchen 1984) and is too steep to support Threespine Stickleback (Gach and Reimchen 1989). Both Rouge and Serendipity lakes are considered to be closed systems; like Boulton Lake, they have no inlet and are maintained by groundwater seepage, and beaver dams prevent immigration into the lakes via their outlets (Reimchen 1984; Deagle et al. 1996). Emigration out of these lakes is possible, however, and the molecular and morphological cline that has been described from Rouge Lake suggests that there is downstream gene flow (lake fish are unarmoured and monomorphic for the Trans-North Pacific lineage while fish at the mouth of its outlet are armoured and monomorphic for the Euro-North American lineage; Deagle et al. 1996).

## Interspecific Interactions

#### The Giant Threespine Stickleback

The Giant Threespine Stickleback is thought to have evolved its distinct morphology at least in part as a result of adaptation to predation by gape-limited fish and birds (see adaptive interpretation of distinguishing morphological characteristics in **Morphological Description** section). Adult Giant Threespine Sticklebacks have a yearly probability near 0.1 of being attacked and escaping from a vertebrate predator (Reimchen 1988), and the long potential life span of this fish greatly extends the period over which predators can exert a selective pressure.

Coastal Cutthroat Trout (*Oncorhynchus clarkii clarkii*) is the major fish predator of the Giant Threespine Stickleback in both Mayer (Moodie 1972b) and Drizzle lakes (Reimchen 1990, 1994). Indeed the Giant Threespine Stickleback is the major food item for this fish (Moodie 1972b; Reimchen 1990). In Mayer Lake, it is followed by the Prickly Sculpin (*Cottus asper*), which preys mainly on juveniles and probably larvae and eggs (Moodie 1972b). These piscivorous fish are limited to Mayer Lake, with none occurring in its streams (Moodie 1972b). Stomach analysis of the non-resident salmonids, Coho

Salmon (Oncorhynchus kisutch) and Dolly Varden (Salvelinus malma), from Mayer Lake found no evidence of stickleback predation by these species (Moodie 1972b). Given that Dolly Varden and Coho Salmon are also found in Drizzle Lake, it is likely that predatory Prickly Sculpin can also access it (Moodie and Reimchen 1976).

Avian Threespine Stickleback predators can be numerous on these lakes. Of the 36 bird species that have been documented on Drizzle Lake, nine were piscivorous foragers that consumed Threespine Stickleback (including resident Common Loon [Gavia immer], Red-necked Grebe [Podiceps grisegena], Horned Grebe [Podiceps auritus], Double-crested Cormorant [Phalacrocorax auritus], Hooded Merganser [Lophodytes cucullatus], Oldsquaw [Clangula hyemalis] and Belted Kingfisher [Ceryle alcyon], and itinerant Common and Red-throated Loons [Gavia stellata]; Reimchen and Douglas 1980, 1984). Of these, the Common Loon accounted for the majority (59 %) of fish consumed by birds (Reimchen and Douglas 1984) and, alongside Coastal Cutthroat Trout, is suspected of exerting a significant evolutionary pressure on the morphology of Threespine Stickleback.

The two predominant predators vary in the size and location of stickleback that they tend to consume, with Coastal Cutthroat Trout being the primary predators of juvenile and subadult stickleback in the littoral zone (Reimchen 1990) and the Common Loon being the primary predator of subadults and adults in the limnetic zone (Reimchen 1994).

# The Unarmoured Threespine Stickleback

In sharp contrast to the lakes containing the Giant Threespine Stickleback, those containing the Unarmoured Threespine Stickleback are considered to have no predatory fish in them. Boulton Lake yielded no other fish samples despite extensive sampling (Moodie and Reimchen 1976; Reimchen 1980), and only Dolly Varden has been found in Rouge Lake (Reimchen 1984). Instead, the Unarmoured Threespine Stickleback is thought to have evolved its distinguishing loss of defensive structures as a result of adaptation to avian and macoinvertebrate predation regimes (see adaptive interpretation of distinguishing morphological characteristics in Morphological Description section).

Seven species of piscivorous birds forage on Boulton Lake in low numbers, with the Common Loon followed by the Belted Kingfisher predominating (other species are Red-necked Grebe, Horned Grebe, Common Merganser (*Mergus merganser*), Red-breasted Merganser (*M. serrator*), and Hooded Merganser (Reimchen 1980).

Common macroinvertebrates in Boulton Lake are trichopteran larvae (Phryganeidae), odonate nymphs (Aeshnidae, Cordulidae, Coenagrionidae), leeches (Hirudidae), and occasional adult diving beetles (Dytiscidae; Reimchen 1980). While the leech may prey upon Threespine Stickleback eggs (Moodie 1972b), the odonate nymphs (Aeshna) prey upon the fish themselves (Reimchen 1980).

Two unusual fish parasites are associated with the Unarmoured Threespine Stickleback. The Unarmoured Threespine Stickleback of Rouge Lake has a symbiotic relationship with an unusual taxon of dinoflagellate parasite that is apparently unique for Threespine Sticklebacks (Reimchen and Buckland-Nicks 1990). In addition, the cestode *Cyathocephalus* is common in some of the Haida Gwaii stickleback populations, including Boulton Lake, yet it is rare elsewhere in western Canada (Reimchen 1982).

The introduced North American Beaver may have impacted the species indirectly via habitat alteration (see **Habitat Trends** and **Population Sizes and Trends** sections).

## POPULATION SIZES AND TRENDS

# **Sampling Effort and Methods**

## The Giant Threespine Stickleback

Mark-recapture methods were used to estimate the adult population size of the Giant Threespine Stickleback in Drizzle Lake in 1985. Estimates were based on 3,803 adult fish that were recaptured in summer from the 17,033 that were marked in the spring using Petersen's and Schnabel's methods (Reimchen 1990). At the same time, visual counts of nests in different littoral areas led to estimates of nest densities and numbers of breeding males (Reimchen 1990). Average clutch size was then used to estimate total recruitment to the lake (Reimchen 1990).

## The Unarmoured Threespine Stickleback

Crude estimates of total population size of the Unarmoured Threespine Stickleback have been derived using several unspecified sampling techniques (Reimchen 1984).

## **Abundance**

# The Giant Threespine Stickleback

Population estimates of adult stickleback ranged from 30,000-120,000 (mean = 75,000) for the Giant Threespine Stickleback in Drizzle Lake in 1985 (Reimchen 1990). Estimates varied depending on the calculation method used (Reimchen 1990). Based on nest density recordings, 10,000-60,000 nests were estimated to occur in the lake over the three-month breeding season (Reimchen 1990). Standard mark-recapture techniques have a number of assumptions, such as closed population, sufficient longevity of marks, equal survival of marked and unmarked individuals, and capture success that is unrelated to presence of a mark or prior capture. Specifically in the case of sticklebacks, these estimates apply to individuals that can be caught with minnow traps. This method of capture may underestimate abundance of adults that tend to be more limnetic in their habits as minnow traps tend to be less effective in pelagic habitats. Nevertheless, this survey was conducted over the breeding season when fish are known to prefer the littoral zone (Moodie 1972a, 1984).

With an average of 395 eggs per nest (range 166-1014, N = 32), recruitment from 30,000 nests would be 12 million fry, assuming 100% survival to hatching. It is likely that the majority of this recruitment would be lost, however e.g., to egg consumption by adult sticklebacks and leeches, and cannibalism of fry by older sticklebacks (Hyatt and Ringler 1989a,b; Reimchen 1990). Based on an estimate of 0.2 % survival of fry to adulthood from another lake in coastal British Columbia (Hyatt & Ringler 1989a), annual fry recruitment in Drizzle Lake would be 24,000. With an average age of approximately 4 years old (Gambling and Reimchen 2012), about four annual recruitment events will accumulate to form a population of about 96,000 adults. This estimate is within the range of the mark-recapture estimates (Reimchen 1990).

No population estimates have been made for the Giant Threespine Stickleback in Mayer Lake. Expert opinion based on general observations of adult stickleback in the littoral zones estimates that the adult population exceeds 100,000 in this larger lake (Moodie 1984; Reimchen 2004).

## The Unarmoured Threespine Stickleback

Total population sizes have been crudely estimated to be 350,000 for Boulton Lake, 17,500 for Rouge Lake, and 22,000 for Serendipity Lake (Reimchen 1984). The Unarmoured Threespine Stickleback from Boulton and Rouge lakes exhibit very low heterozygosity (< 0.1 from genome-wide SNP analyses; Jones *et al.* 2012). They do, in fact, have amongst the lowest levels recorded for freshwater populations surveyed from across the *G. aculeatus* range (Jones *et al.* 2012). Given the small size of Boulton and Rouge lakes (< 20 ha), it is very likely that small effective population size, perhaps as well as demographic histories involving bottlenecks during colonization, have contributed to this low genetic diversity.

## **Fluctuations and Trends**

## The Giant Threespine Stickleback

There has been no systematic monitoring of abundance of the Giant Threespine Stickleback in either Mayer or Drizzle lakes, so population trends are unknown. However, general observations and the ease with which adult stickleback can be caught in the littoral zones of Mayer Lake indicate no qualitative evidence of change in abundance since research began in the late 1960s (Moodie 1984; Reimchen 2004). Population density is likely regulated, at least in part, by the availability of spawning sites and predator abundance (Moodie 1984). Because there has been no account of change in either of these (see **Habitat Trends** and **Interspecific Interactions** sections), this limited information suggests that population size is stable (Moodie 1984).

# The Unarmoured Threespine Stickleback

There has been no systematic monitoring of abundance of the Unarmoured Threespine Stickleback in either Boulton, Rouge, or Serendipity lakes. While there were no obvious changes in abundance during earlier sampling periods (Boulton 1970-81; Rouge 1976-81; Serendipity 1979-81) based on trap success (number of fish per trap hour; Reimchen 1984), population trends in more recent decades are unknown.

Rouge and Serendipity lakes underwent a period of habitat alteration due to beaver activity around the earlier sampling time (see **Habitat Trends** section; Reimchen 1984). The impact of these changes on fish abundance have not been documented but rising water levels have the potential to change recruitment rates by decreasing nesting areas, disrupting mate recognition, and increasing lake use by predatory birds such as loons (Reimchen 1984). Because water levels appear to have stabilized after that time (Reimchen 1984) and there have been no further accounts of change, this limited information suggests that population size is stable.

#### **Rescue Effect**

The concept of rescue effect does not apply to the Canadian population of either the Giant or Unarmoured Threespine Stickleback; each is composed of a single DU and has a global range restricted to only two or three lakes within Canada.

## THREATS AND LIMITING FACTORS

Age-structured population modelling suggests that Threespine Stickleback are resilient to habitat disturbances (Hatfield 2009). Nevertheless, their short life span contributes to their vulnerability to specific threats, such as nest predation. This is evidenced by the rapid extinction of the Hadley Lake Benthic and Limnetic Sticklebacks by an invasive catfish, the Brown Bullhead (*Ameiurus nebulosus*; Hatfield 2001), and the creation of a hybrid swarm in the Enos Lake Benthic and Limnetic Sticklebacks following the introduction of the American Signal Crayfish (*Pascifasticus leniusculus*; Taylor *et al.* 2006). Invasive species are a known, ongoing threat to freshwater fishes and their habitats, and present the largest threat to both the Giant and Unarmoured Threespine Stickleback (Rosenfeld pers. comm. 2013).

Both of these forms have narrow environmental specificity. In summary, the distinguishing phenotype of the Giant Threespine Stickleback most likely depends on selective pressure exerted from gape-limited predators (predominantly Coastal Cutthroat Trout and loons). Like other Threespine Stickleback, it also needs gently sloping sand/gravel beaches and natural littoral macrophytes for nesting and juvenile rearing. The Unarmoured Threespine Stickleback is limited to small, shallow, acidic bog ponds that have no predatory fish and few avian predators (see **Habitat Requirements** and **Interspecific Interactions** section). Alterations to these specific habitat requirements by anthropogenic disturbance most likely present another significant threat to both forms. Specific threats are outlined below for each DU. Completion of the IUCN Threat Assessment worksheet for both sticklebacks returned overall threat levels of "Low" (Appendices).

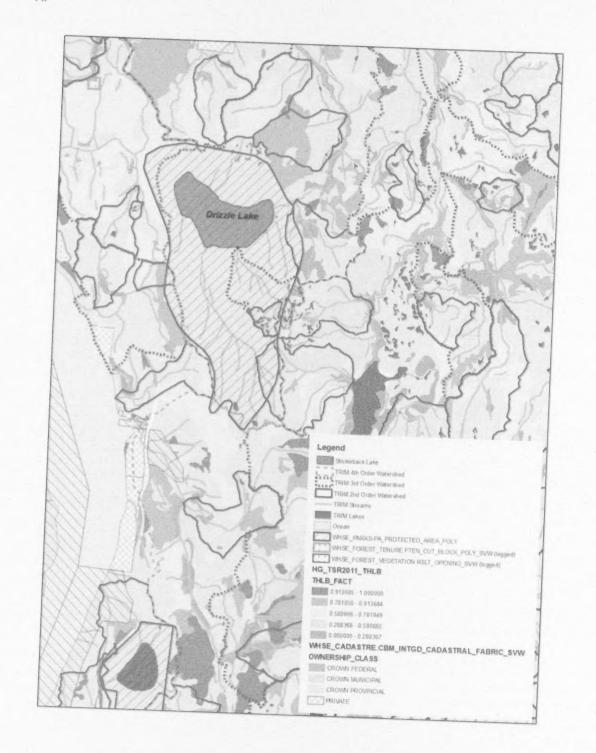
## The Giant Threespine Stickleback

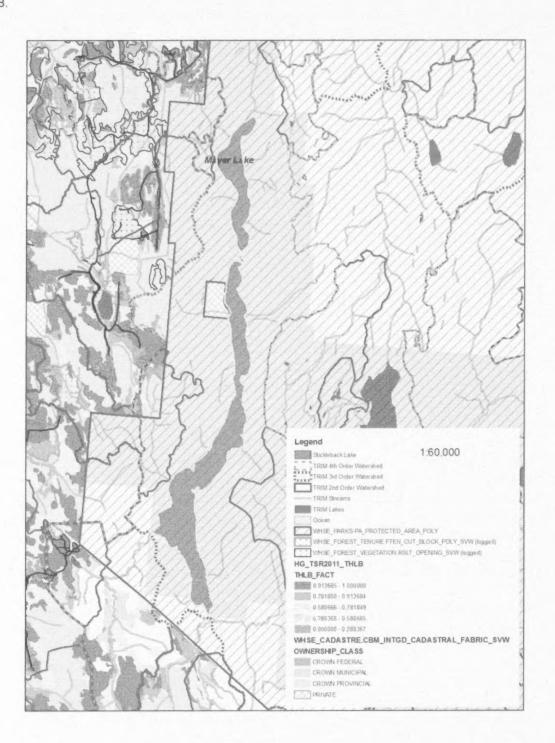
Given that predation of the Giant Threespine Stickleback by gape-limited predators is most likely a key selective force in the maintenance of its distinct morphology, a decline in predation pressure from Coastal Cutthroat Trout and/or Common Loon could lead to adaptive alterations of phenotype and genetic structure, including hybridization with their "typical" parapatric stream counterparts (Moodie 1984). Consequently, the predator regimes of Mayer and Drizzle Lakes need to be protected. In particular, Coastal Cutthroat Trout need protection from overfishing and loons from excessive disturbance (Moodie 1984).

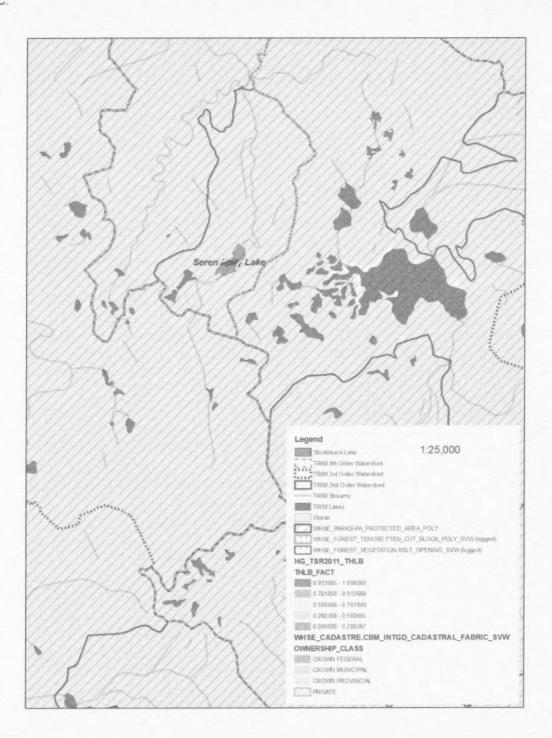
Growing recreational pressure from increased tourism on Haida Gwaii has the potential to increase the risk of these threats, as well as the risk of introducing bait fish and non-native game fish that could in turn alter the predator regime in Mayer and Drizzle lakes (Moodie 1984). The consequences of invasive species introductions can be catastrophic for Threespine Stickleback (e.g., see discussion in Taylor et al., 2006). The probability of introductions on Haida Gwaii is, however, probably lower than for many other Threespine Stickleback lakes, given the relative isolation of Haida Gwaii from many sources of invasive species, and its projected stable human population size (BC Stats 2013). Nevertheless, accessibility of lakes harbouring Giant Threespine Stickleback could increase this risk: Drizzle Lake is accessible only by foot, lying 1.6 km from the Haida Gwaii Highway, but Mayer Lake is the most accessible lake on Haida Gwaii, with vehicle access from the Haida Gwaii Highway. Furthermore, various risk assessments have been completed for BC freshwaters (e.g., Bradford et al. 2008; Tovey et al. 2008) have indicated that at least some lakes on Haida Gwaii have environmental conditions that are suitable for non-native predatory fishes such as Smallmouth Bass (Micropterus dolomieu) and Northern Pike (Esox lucius). There is, however, some capacity to manage these potential threats: Drizzle Lake lies within the protective confines of Drizzle Lake Ecological Reserve and Mayer Lake lies within Naikoon Provincial Park. It is unknown whether current angling restrictions and enforcement practices are successfully mitigating against this concern.

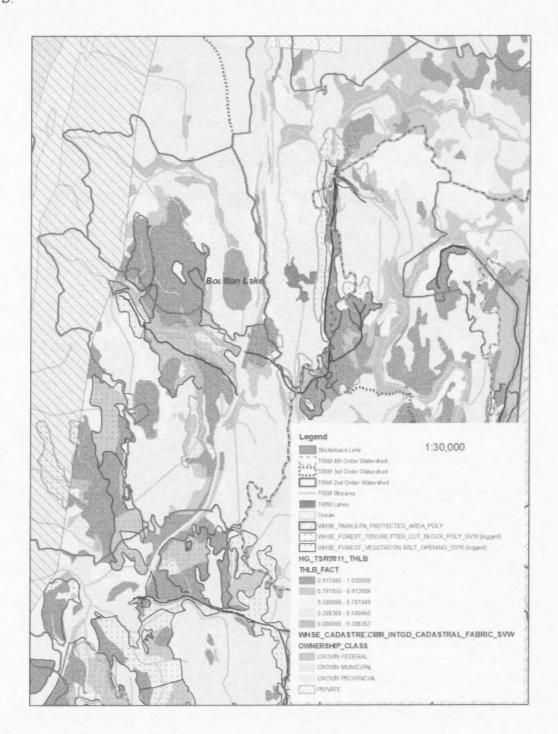
These protected areas also afford Mayer and Drizzle Lake some protection from logging activities. The entire catchment of Drizzle Lake is contained within the boundaries of the Drizzle Lake Ecological Reserve, and so it is considered to have "negligible risk" from future harvest potential (Figure 7a, Cober pers. comm. 2013). Although Mayer Lake has extensive watershed catchment in Naikoon Provincial Park, forestry operations could impact some drainages that flow into the lake, so it is considered to have "some risk" (Figure 7b, Cober pers. comm. 2013).

The North American Beaver has some potential to flood shorelines and disrupt native vegetation. This could restrict the availability of spawning sites in the littoral zone (BCMWLAP 2004), a probable limiting factor for Threespine Stickleback. There has, however, been no observable impact of beaver activity on any sticklebacks since its introduction to Haida Gwaii in the middle of the twentieth century. Combined with the relatively large size of the Giant Threespine Stickleback lakes, it seems unlikely that beaver activity would be detrimental to the Giant Threespine Stickleback.









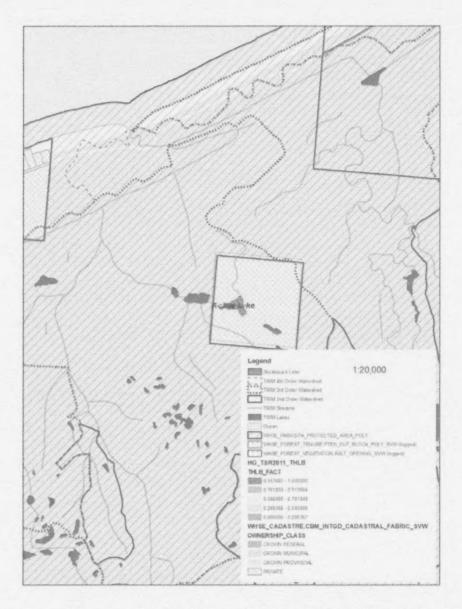


Figure 7. Assessment of potential risk from future harvest <u>potential</u> in the watershed catchments of the Giant and Unarmoured Threespine Sticklebacks. Key inputs are: watershed basins (2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup> order); protected areas (WHSE\_PARKS-PA\_PROTECTED\_AREA\_POLY); ownership class (WHSE\_CADASTRE.CBM\_INTGD\_CADASTRAL\_FABRIC\_SVW); harvest history to date (WHSE\_FOREST\_VEGETATION.RSLT\_OPENING\_SVW and WHSE\_FOREST\_TENURE.FTEN\_CUT\_BLOCK\_POLY\_SVW); most recent timber supply review process for Haida Gwaii [2011 process leading to 2012 AAC decision] (HG\_TSR2011\_THLB) classified into five categories for the forested polygons, the operable land base ranging from probably never contributing to timber supply (0.000000 - 0.288367) to contributing to timber supply in a substantive way (0.913685 - 1.000000). A. Drizzle Lake; B. Mayer Lake; C. Serendipity Lake; D. Boulton Lake; E. Rouge Lake. Source: Cober (pers. comm. 2013).

## The Unarmoured Threespine Stickleback

The Unarmoured Threespine Stickleback's lack of armour structures that typically defend Threespine Stickleback from gape-limited predators make them particularly susceptible to the introduction of any predatory fish (Reimchen 1984). The establishment of predatory fish, such as Coastal Cutthroat Trout which are native to the Haida Gwaji archipelago, for sport fishing would change the selective regime of the lakes the Unarmoured Threespine Stickleback inhabits. This could potentially eliminate the Unarmoured Threespine Stickleback by diminishing its population size and/or drastically altering its genetic structure. The capacity for its small, shallow lakes to harbour such fish is supported by the occurrence of other small (< 10 ha) and shallow (< 10 m) lakes in coastal British Columbia that are known to sustain fish other than Threespine Stickleback, including Coastal Cutthroat Trout (Ormond et al. 2011). Growing recreational pressure from increased tourism on Haida Gwaii has the potential to increase the risk of artificial introduction of sports fish, as does the close proximity of Boulton Lake to the Haida Gwaii Highway (it is within walking distance of this major road). As for the Giant Threespine Stickleback, risk assessments suggest that suitable habitats for invasive predatory fishes exist on Haida Gwaii (e.g., Bradford et al. 2008; Tovey et al. 2008).

Serendipity Lake is thought to have "negligible risk" from future harvest potential as its entire catchment is contained within Naikoon Provincial Park (Figure 7c, Cober pers. comm. 2013). In contrast, Boulton and Rouge lakes could be at increased risk from rural and industrial activities, such as real estate development, agriculture and logging activities (Reimchen 1984). Indeed, Boulton Lake is considered to have "some risk" from future harvest potential due to a portion of its catchment area lying in operable land base (Figure 7d, Cober pers. comm. 2013). It lies on Crown land, and is only afforded protection by the Haida Gwaii Land Use Objectives Order that provides and reserve a narrow riparian buffer around it (a two-tree-length provision based on average stand height). The topographic setting of Boulton, which sits near the top of its limited watershed catchment area, makes it potentially vulnerable to e.g., oils spills from any adjacent logging or perhaps even from the east side of the Haida Gwaii Highway (Cober pers. comm. 2013). The risk to Rouge Lake from future harvest potential is more difficult to predict, given that much of its catchment lies within a private holding that is itself an in-holding of Naikoon Provincial Park (Figure 7e, Cober pers. comm. 2013).

Habitat change due to introduced beaver activity has the potential to negatively influence the Unarmoured Threespine Stickleback subpopulations. For example, rising water levels could change recruitment rates by decreasing nesting areas, disrupting mate recognition, and increasing use of the lake by predatory birds such as loons (Reimchen 1984). Nevertheless, no impact on subpopulations was described for either Rouge or Serendipity lakes when beaver activity altered habitat several decades ago (see **Habitat Trends** and **Populations Sizes and Trends** sections; Reimchen 1984) so the imminence of this threat seems low.

Winter kill from severe winter conditions is of concern to the Unarmoured Threespine Stickleback as it inhabits small lakes (Reimchen 1984). The fish, however, have successfully persisted for at least several thousand years so the imminence of this threat seems low. Likely, a more pressing climatic threat for Serendipity Lake arises from its relatively low elevation (20 m, Johnson and Taylor 2004) and its close proximity to the east beaches of Graham Island, which are known to be vulnerable to erosion caused by climate change and sea-level rises (Walker *et al.* 2007). In the future, Serendipity Lake may well be at risk of drainage caused by coastal erosion (Cober pers. comm. 2013).

# PROTECTION, STATUS AND RANKS

# **Legal Protection and Status**

COSEWIC designated the Giant and Unarmoured Threespine Sticklebacks as Special Concern in April 1980 and 1983, respectively. As such, the Species at Risk Act (SARA) status of these species is currently Schedule 3, Special Concern. Species that were designated at risk by COSEWIC prior to October 1999, however, must be reassessed against revised criteria before they can be considered for addition to Schedule 1 of SARA.

Recent changes to the *Fisheries Act* result in habitat protection provisions that will only apply to fishes that are the focus of commercial, recreational, or Aboriginal fisheries, which does not include the Giant and Unarmoured Threespine Sticklebacks. The *Fisheries Act* also delegates authority to the provinces and territories to establish and enforce fishing regulations. In accordance with this Act, the *BC Sport Fishing Regulations* stipulate that it is illegal to fish for, or catch and retain either of the Giant or the Unarmoured Threespine Stickleback (DJC 1996). The Giant and Unarmoured Threespine Stickleback are also afforded some protection in British Columbia under the Canadian federal *Wildlife Act*, which enables provincial and territorial authorities to license anglers and angling guides, and to supply scientific fish collection permits.

# Non-Legal Status and Ranks

The Giant Threespine Stickleback has a Global Heritage Status rank of Critically Imperilled (G1, NatureServe 2012), meaning that it is considered to be at very high risk of extinction across its entire range. It is also listed as Critically Imperilled nationally in Canada (N1) and subnationally in British Columbia (S1; NatureServe 2012). Its General Status at the Canada and provincial levels was listed as Sensitive in 2000 (Wild Species 2011). It is "red-listed" by the Conservation Data Centre and BC Ministry of Environment (BCCDC 2012). Under the BC Conservation Framework, the Giant Threespine Stickleback is ranked 1 (highest priority) under Goal 1 (Contribute to global efforts for species and ecosystem conservation) and Goal 3 (Maintain the diversity of native species and ecosystems; BCCF 2012a).

The Unarmoured Threespine Stickleback has not yet been assigned a Global Heritage Status rank (NatureServe 2012). It is listed as Imperilled nationally in Canada (N2) and subnationally in British Columbia (S2; NatureServe 2012). This indicates that it is considered to be at high risk of extinction. Its General Status at the Canada and provincial levels has not been assessed (Wild Species 2011). It is "red-listed" by the Conservation Data Centre and BC Ministry of Environment (BCCDC 2012). Under the BC Conservation Framework, the Unarmoured Threespine Stickleback is ranked 1 (highest priority) under Goal 1 (BCCF 2012b).

## **Habitat Protection and Ownership**

Most of the habitat of the Giant Threespine Stickleback is afforded some level of protection from development. The Drizzle Lake watershed (837 ha) was established as an Ecological Reserve in 1973, principally to maintain the ecosystem for research on the Giant Threespine Stickleback and their associated predators (BCMWLAP 2004). Consumptive uses such as hunting, fishing, camping and grazing, or removal of materials, plants or animals are prohibited. Mayer Lake and much of its drainage basin occurs within the boundary of Naikoon Provincial Park. Although camping and recreational fishing are allowed here, rural and industrial development, such as real estate development and logging activities, are prohibited.

Rouge and Serendipity Lake watersheds, which harbour the Unarmoured Threespine Stickleback, are also located within the boundary of Naikoon Provincial Park. Rouge Lake is located on a 130ha private holding within this park. A 70ha Ecological Reserve has been proposed for Boulton Lake watershed (PMT HG/QCI LUPP 2006). As it lies on Crown land, its fish habitat is afforded some protection from forestry activities from the *BC Forest and Range Practices Act*.

#### ACKNOWLEDGEMENTS AND AUTHORITIES CONTACTED

The report writer would like to extend her gratitude to all of the following people for generously sharing information and advice (see also Table 2): Eric Taylor (University of British Columbia); Daniel Berner (Universität Basel); Bruce Deagle and Tom Reimchen (University of Victoria); Christie Whelan, Sean MacConnachie (Department of Fisheries and Oceans); Patrick Nantel (Parks Canada); Gregory Wilson (BC Ministry of Environment), Alvin Cober (BC Ministry of Environment), and Katrina Stipec (British Columbia Conservation Data Centre). The report writer would also like to thank Jenny Wu (COSEWIC Secretariat) for her collegiality in creating the distribution map and calculating extent of occurrence and area of occupancy. Neil Jones (COSEWIC) informed the author that the status of ATK information for the Giant and Unarmoured Threespine Sticklebacks is unknown at the time of submission of this report.

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# BIOGRAPHICAL SUMMARY OF REPORT WRITER(S)

Jennifer Gow, M.Res, PhD, is the Deputy Managing Editor of Molecular Ecology and Molecular Ecology Resources. She has worked in the field of conservation biology and molecular ecology for more than a decade. Jennifer began applying her expertise in molecular ecology to Threespine Stickleback native to British Columbia in 2003, when she started postdoctoral work at the University of British Columbia. Her research there has given insight into the ecological and evolutionary forces that shape patterns of genetic diversity in these and other freshwater fishes native to Canada.

#### COLLECTIONS EXAMINED

No collections were examined for this report.

# Appendix 1. Threats Assessment Worksheet for the Giant Threespine Stickleback.

Species or Ecosystem	Giant Threespin	e Stickleback			
Element ID		Elcode			
Date (Ctrl + ";" for today's date):	16/08/201 3				
Assessor(s):	E. Taylor	Targata			
References:					
Overall Threat Impact Calculation Help:			Level 1 Threat Impact Counts		
	Threat Impact		high range	low range	
	A	Very High	0	0	
	В	High	. 0	0	
	C	Medium	0	0	
	D	Low	0	0	
	Calculated Over	rall Threat impact:			
	Assign	ned Overall Threat	D = Low		
	Impact Adi	Impact: ustment Reasons:			Anna Anna Anna Anna Anna Anna Anna Anna
		Threat Comments	Remote locations on	Haida Gwaii and	within ecological
	Overan	mea confinence	reserves and provinc populations. Threat of species is low, but co	ial park offer son f introduction of	ne secruity for exotic aquatic

Threat		Impact (calculated)	Scope (next 10 Yrs)	Severity (10 Yrs or 3 Gen.)	Timing	Comments	
1	Residential & commercial development	Negligible	Negligible(<1%)	Negligible (<1%)	Low (Possibly in the long term, >10 yrs)		
1.1	Housing & urban areas	Negligible	Negligible(<1%)	Negligible (<1%)	Low (Possibly in the long term, >10 yrs)		
1.2	Commercial & industrial areas	Negligible	Negligible(<1%)	Negligible (<1%)	Insignificant/Negligible (Past or no direct effect)		
1.3	Tourism & recreation areas	Negligible	Negligible(<1%)	Negligible (<1%)	Low (Possibly in the long term, >10 yrs)		
2	Agriculture & aquaculture						
2.1	Annual & perennial non-timber crops						
2.2	Wood & pulp plantations						

Thre	Threat  2.3 Livestock farming & ranching		spact Scope (next		Severity (10 Yrs or 3 Gen.)	Timing	Comments
2.3							
2.4	Marine & freshwater aquaculture						
3	Energy production & mining						
3.1	Oil & gas drilling						
3.2	Mining & quarrying						
3.3	Renewable energy						
4	Transportation & service corridors		Negligible	Negligible(<1%)	Negligible (<1%)	Insignificant/Negligible (Past or no direct effect)	
4.1	Roads & railroads		Negligible	Negligible(<1%)	Negligible (<1%)	Low (Possibly in the long term, >10 yrs)	
4.2	Utility & service lines						
4.3	Shipping lanes						
4.4	Flight paths						
5	Biological resource		Negligible	Small(1-10%)	Negligible (<1%)	Insignificant/Negligible (Past or no direct effect)	
5.1	Hunting & collecting terrestrial animals						
5.2	Gathering terrestrial plants						
5.3	Logging & wood harvesting						
5.4	Fishing & harvesting aquatic resources		Negligible	Small(1-10%)	Negligible (<1%)	Low (Possibly in the long term, >10 yrs)	
6	Human intrusions & disturbance						
6.1	Recreational activities		Negligible	Small(1-10%)	Negligible (<1%)	Low (Possibly in the long term, >10 yrs)	
6.2	War, civil unrest & military exercises						
6.3	Work & other activities						Park maintenance activities at Tlell, dune dynamic research work (although this study group is well aware of species at risk)

Threat		(calculated)		Scope (next 10 Yrs)	Severity (10 Yrs or 3 Gen.)	Timing	Comments
7	Natural system modifications		Negligible	Small(1-10%)	Negligible (<1%)	Low (Possibly in the long term, >10 yrs)	
7.1	Fire & fire suppression		Will Will				
7.2	Dams & water management/use						
7.3	Other ecosystem modifications		Not Calculated (outside assessment timeframe)	Unknown	Unknown	Low (Possibly in the long term, >10 yrs)	Introduced beaver could alter limnology, habitat structure (shoreline extent), changes in predation pressure from trout and loons if population sizes of those native predators change
8	Invasive & other problematic species & genes		Not Calculated (outside assessment timeframe)	Pervasive (71-100%)	Extreme (71-100%)	Low (Possibly in the long term, >10 yrs)	Probability of introduction of invasives is small owing to remote locations, but consequences likely severe as has been documented in other stickleback populations
8.1	Invasive non- native/alien species		Not Calculated (outside assessment timeframe)	Pervasive (71- 100%)	Extreme (71-100%)	Low (Possibly in the long term, >10 yrs)	
8.2	Problematic native species						
8.3	Introduced genetic material						
9	Pollution						
9.1	Household sewage & urban waste water						
9.2	Industrial & military effluents						
9.3	Agricultural & forestry effluents						
9.4	Garbage & solid waste						
9.5	Air-borne pollutants						
9.6	Excess energy						
10	Geological events						
10.1	Volcanoes		Barrier .				
10.2	Earthquakes/ tsunamis	D	Low	Small(1-10%)	Moderate (11-30%)	Moderate (Possibly in the short term, < 10 yrs)	
10.3	Avalanches/landslid es						
11	Climate change & severe weather						

Threat		Impact (calculated)				Timing	Comments	
11.1	Habitat shifting & alteration							
11.2	Droughts							
11.3	Temperature extremes							
11.4	Storms & flooding							

Classification of Threats adopted from IUCN-CMP, Salafsky et al. (2008).

# Appendix 2. Threats Assessment Worksheet for the Unarmoured Threespine Stickleback.

Species or Ecosystem	Unarmoured	Threespine Sti	ckleback			
Element ID			Elcode			
Date (Ctrl + ";" for today's date):	05/09/2013					
Assessor(s):	E. Taylor		3,511,511,511,511			
References:						
Overall Threat Impact Calculation Help:				Level 1 Threat		
	Threat Impact			high range	low range	
	A	Very High		0	0	
	В	High		0	0	
	C	Medium	NAME OF THE OWNER.	0	0	
	D	Low		1	1	
		Calculate	d Overall Threat Impact:	Low	Low	
			d Overall Threat Impact:	D = Low		
		Im	pact Adjustment Reasons:			
		Overall Ti	hreat Comments	Remote locations or within a provincial particle. Threat of introduction consequences would	ark offer some secru in of exotic aquatic s	

Threat		Impact (calculated)		Severity (10 Yrs or 3 Gen.)	Timing	Comments
1	Residential & commercial development	Negligible	Negligible(<1 %)	Negligible (<1%)	Low (Possibly in the long term, >10 yrs)	Boulton and Rouge lakes potentially susceptible
1.1	Housing & urban areas	Negligible	Negligible(<1 %)	Negligible (<1%)	Low (Possibly in the long term, >10 yrs)	
1.2	Commercial & industrial areas	Negligible	Negligible(<1 %)	Negligible (<1%)	Insignificant/Negligible (Past or no direct effect)	
1.3	Tourism & recreation areas	Negligible	Negligible(<1 %)	Negligible (<1%)	Low (Possibly in the long term, >10 yrs)	
2	Agriculture & aquaculture					
2.1	Annual & perennial non- timber crops					
2.2	Wood & pulp plantations					
2.3	Livestock farming & ranching					
2.4	Marine & freshwater aquaculture					
3	Energy production & mining					
3.1	Oil & gas drilling					

Thre	eat	Impact Scope (next 10 Yrs)		Severity (10 Yrs or 3 Gen.)	Timing	Comments
3.2	Mining & quarrying					
3.3	Renewable energy					
1	Transportation & service corridors	Negligible	Negligible(<1 %)	Negligible (<1%)	Insignificant/Negligible (Past or no direct effect)	
4.1	Roads & railroads	Negligible	Negligible(<1 %)	Negligible (<1%)	Low (Possibly in the long term, >10 yrs)	
4.2	Utility & service lines					
4.3	Shipping lanes					
4.4	Flight paths					
5	Biological resource use	Negligible	Small(1-10%)	Negligible (<1%)	Insignificant/Negligible (Past or no direct effect)	
5.1	Hunting & collecting terrestrial animals					
5.2	Gathering terrestrial plants					
5.3	Logging & wood harvesting					
5.4	Fishing & harvesting aquatic resources	Negligible	Small(1-10%)	Negligible (<1%)	Low (Possibly in the long term, >10 yrs)	
3	Human intrusions & disturbance					
6.1	Recreational activities	Negligible	Negligible(<1 %)	Negligible (<1%)	Low (Possibly in the long term, >10 yrs)	
6.2	War, civil unrest & military exercises					
5.3	Work & other activities					
7	Natural system modifications	Negligible	Small(1-10%)	Negligible (<1%)	Low (Possibly in the long term, >10 yrs)	
7.1	Fire & fire suppression					
7.2	Dams & water management/use					
7.3	Other ecosystem modifications	Not Calculated (outside assessment timeframe)	Unknown	Unknown	Low (Possibly in the long term, >10 yrs)	Small lakes make them susceptible; introduced beave could alter limnology, habitat structure (shoreline extent)
3	Invasive & other problematic species & genes	Not Calculated (outside assessment timeframe)	Pervasive (71- 100%)	Extreme (71-100%)	Low (Possibly in the long term, >10 yrs)	
3.1	Invasive non-native/alien species	Not Calculated (outside assessment timeframe)	Pervasive (71- 100%)	Extreme (71-100%)	Low (Possibly in the long term, >10 yrs)	Probability of introduction of aquatic invasives is low, but consequences have been severe in other stickleback populations. Habitats are suitable for some invasives
3.2	Problematic native species					The state of the s
3.3	Introduced genetic material					
9	Pollution					

Threat		impact (calculated)	Scope (next 10 Yrs)	Severity (10 Yrs or 3 Gen.)	Timing	Comments
9.1	Household sewage & urban waste water	Not Calculated (outside assessment timeframe)	Small(1-10%)	Slight (1- 10%)	Low (Possibly in the long term, >10 yrs)	Boulton and Rouge lakes could experiences some residential developments in watershed
9.2	Industrial & military effluents					
9.3	Agricultural & forestry effluents	Not Calculated (outside assessment timeframe)	Small(1-10%)	Slight (1- 10%)	Low (Possibly in the long term, >10 yrs)	Boulton Lake has forestry potential in watershed; Rouge Lake some agricultural and industrial developments
9.4	Garbage & solid waste					
9.5	Air-borne pollutants					
9.6	Excess energy					
10	Geological events					
10.1	Volcanoes					
10.2	Earthquakes/ tsunamis	Not Calculated (outside assessment timeframe)	Small(1-10%)	Moderate (11-30%)	Low (Possibly in the long term, >10 yrs)	
10.3	Avalanches/landslides					
11	Climate change & severe weather					
11,1	Habitat shifting & alteration					
11.2	Droughts					
11.3	Temperature extremes					
11.4	Storms & flooding	Not Calculated (outside assessment timeframe)	Small(1-10%)	Serious (31- 70%)	Low (Possibly in the long term, >10 yrs)	Serendipity Lake susceptible to coastal erosion from climate change

Classification of Threats adopted from IUCN-CMP, Salafsky et al. (2008).